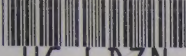


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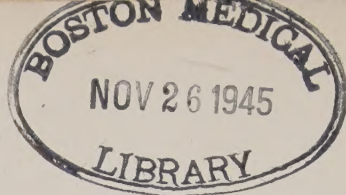
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A CASE OF ILIOTHORACOPAGUS (DICEPHALUS TRIBRACHIUS TRIPUS) WITH A CONSIDERATION OF THE "BUDDING" AND "FISSION" THEORIES OF TWINNING

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THREE PLATES (SIX FIGURES)

The case to be presented is that of conjoined, female infants who were born prematurely at the end of the seventh lunar month, who died soon after birth and whose combined weight after preservation in formalin was 2016 gm. (fig. 1).¹ This form of equal but incomplete twinning should be classified as Perpendicular Symmetrical Duplication (dicephalus tribrachius tripus) or as Supra- and Infra-umbilical Vento-lateral Union (iliothoracopagus); see Fisher (1866), Schwalbe ('07), Hubner ('11) and Scammon ('24). Although there are at least twelve similar cases in the literature, only one of them has been studied by means of adequate dissection, namely Röver's case ('37).²

In order to facilitate description and discussion, the right twin (the one on the left in fig. 1) will be referred to as Twin I and the left as Twin II. Likewise certain structures and their embryonic primordia will be designated either I or II.

¹The specimen was received from Dr. E. G. Oppen, Minneapolis, who kindly supplied the following clinical information. The mother was a primipara, aged 24, and her Wasserman test was negative. Because of placenta previa, vaginal bleeding occurred before hospitalization. In spite of progesterone and sedatives, premature delivery was spontaneous. The menstrual age of the twins was 197 days. Insofar as the mother could recall the first month of her pregnancy, there were neither abnormal symptoms nor complicating events.

²Röver's case differs from ours by exhibiting such anomalies as horseshoe kidney, absence of one umbilical artery, presence of two superior venae cavae in the left twin and passage of the left subclavian artery behind the esophagus in the right twin. Also, his case fails to show an anomalous right subclavian artery in the left twin. The other eleven cases are as follows. Eight are those collected from the literature by Röver; of them, it was possible to review five, namely those of Agaronian ('34). Batnew (case 1, '00), Kasprzak ('10) and Schwalbe (two cases, '07). In addition to the cases listed by Röver, there are those of Benedini (1844). Reaves ('39) and Wyman (1866).

OBSERVATIONS

1. External and skeletal characteristics

Most of the external characteristics of the twins are shown in figures 1 and 2. Deserving special mention are the single umbilical cord, the clubbing of the feet and the primordia of two mammary glands.³

In the perineal region, only one anus was present and it was situated between a vulva and a second orifice which was exclusively urethral. The vulva presented a normal appearance while the urethral orifice was overhung by a hinged nubbin of tissue (phallus ?).

Figure 3 shows that the duplication of the skeleton was not complete. The two adjacent scapulae were fused. Extending upward from them was a single spike of bone which represented conjoined humeri. The evidence for this was secured by dissection during which it was found that an incomplete set of scapular muscles was attached to this spike-like bone and that there were no muscles distal to it (e.g., no muscles of conjoined forearms). The sternum was broad and it articulated with the right clavicle of Twin I and the left clavicle of Twin II. The remaining two clavicles were adjacent and parallel, their medial ends fusing with the top of the sternum by means of cartilaginous plates. Two sets of costal cartilages articulated with the sternum, while the other two sets (left set of Twin I and right set of Twin II) united with a vertical plate of cartilage which did not otherwise resemble a sternum. There was an extensive cartilaginous union of the left ilium of Twin I with the right ilium of Twin II. The third inferior extremity presented five bones. The first and longest of these appears at the lower left of figure 3, while the second is circular and is situated at the inferior end of the first. The remaining three resemble metatarsals and cast shadows on the proximal third of that femur which lies in the horizontal plane of the photograph.

In figure 2, the point of iliac union is above the base of the third inferior extremity. A vertical line bisecting upper and lower conjoined appendages likewise bisects rudimentary thoracic and abdominal walls. It was possible to dissect and identify most of the muscles

³ A third mammary primordium could not be found. If present, one should expect to find it below the third upper appendage (rudimentary conjoined appendages) because in the normal embryo the mammary ridge develops along a curved line which connects upper and lower limb buds and because in Twins I and II the adjacent embryonic limb buds probably originated as conjoined structures. Incidentally, it would be incorrect to refer to the mammary primordia as nipples since normally the nipples do not appear until several months after birth (Scammon, '23).

composing these walls in spite of the distorted relations and the thinness of the muscles. The muscles of the conjoined appendages could be identified only by means of their skeletal attachments because the muscles presented bizarre shapes and because the pattern of nerves did not assist identification — the two plexuses uniting to form a single trunk from which branches passed to the several muscles. Although these skeletal attachments could be more readily determined in the upper appendage than in the lower, it was still impossible to identify seven of the seventeen paired muscles which might be expected to have attachments on the conjoined scapulae: namely the omohyoideus, supraspinatus, subscapularis, pectoralis minor, biceps brachii, coracobrachialis and triceps brachii.

2. *Thoracic viscera*

After opening the thoracic cavity, one pericardial and four pleural sacs were observed. The four lungs were normal in appearance, and four phrenic nerves supplied the conjoined diaphragms. The pericardial sac enclosed two hearts which were firmly conjoined (fig. 5). In the ventricular region the cardiac union was wall to wall; but above the coronary sulcus, the four atria were telescoped into one inseparable chamber.

Despite first impression to the contrary, the hearts were not mirror images of each other. This was shown by the fact that the pulmonary trunk of Twin I passed behind the aorta (cf. fig. 5a) after emerging from the right ventricle.

The ventricles of Heart II were more nearly normal than those of Heart I. The former were separated by a complete interventricular septum, while the latter communicated with each other by means of a large interventricular foramen.

The common atrium consisted of two chambers of unequal size, one being three times as large as the other. The smaller chamber, being the left atrium of Twin II, received a single pulmonary vessel into which emptied right and left pulmonary veins of this twin. It communicated with the larger chamber by means of an oval foramen, 7.5 mm. in diameter. The larger chamber, fundamentally three chambers in one, was traversed by a vertical cord of tissue that undoubtedly represented a partial interatrial septum of Heart I. However, from the relation of this cord to the atrio-ventricular ostia and to the veins that entered the larger chamber, it was impossible to identify right and left atria of

Heart I. Consequently, it was decided to label the auricular appendages of Heart I on the basis of their proximity to the ventricles.

Four sizeable veins entered the larger atrial chamber. The largest, representing fused inferior venae cavae, pierced the diaphragm (fig. 6) and entered the bottom of the chamber. A second vein, the superior vena cava of Twin II, opened obliquely into the top. The third represented the persisting left common cardinal vein of Twin I. Notable tributaries of this vein were the pulmonary veins and a vessel that belonged to the hemiazygos system of veins. It would seem that during development the pulmonary veins had failed to reach the embryonic atrium and, instead, had continued to communicate with the cardinal system of veins. Undoubtedly this must have occurred at a very early embryonic stage "in which the developing foregut, trachea and lung buds are supplied by a common plexus of small channels that thread in all directions through the loose mesenchyme and communicate with the primitive cardinal veins freely in many places" (Patten, '42). Presumably later development included (1) expansion of certain of these channels (left supracardinal) to accommodate the pulmonary veins and (2) persistence of the terminal segment of the left posterior cardinal vein. The fourth vein entering the larger atrial chamber was an anomalous right superior vena cava of Twin I. The developmental explanation of this vessel is that during the embryonic period no transverse anastomosis developed between right and left thymico-thyroid plexuses or, in other words, that the left anterior cardinal vein failed to communicate with the right.⁴

In Twin II, an anomalous right subclavian artery originated from the back of the aorta and passed behind the esophagus. After giving off the usual branches, the largest being the vertebral, the anomalous artery joined the left subclavian artery of Twin I to produce an "axillary" artery. The latter vessel supplied the third superior extremity. The right inferior laryngeal nerve did not loop around the anomalous artery. Instead, it came off the vagus above the inferior thyroid artery and passed obliquely upwards without looping. Therefore it is probable that in the development of the anomalous subclavian artery the em-

⁴It was impossible to make a satisfactory dissection of the veins which drained the walls of the hearts. Theoretically, the large atrial chamber should have received a coronary sinus from Twin II. Also, since the left anterior cardinal and left common cardinal veins of Twin I had persisted (fig. 5), it is reasonable to suppose that the cardiac veins of Twin I opened into the left common cardinal as in normal embryos. The four coronary arteries, however, were easily followed from the sinuses of the aortae.

bryonic vessels which had dropped out were the right fourth aortic arch and the right aorta down as far as the sixth segmental artery.⁵

The most striking derivative of the aortic arches of Twin I, from the standpoint of mirror imaging, was the anomalous right aorta. It represented the persisting right fourth arch and the right aorta of the embryo.

As indicated in figure 5a, it seems that in Twin I aortic arches 3, 4 and 6 had persisted on both sides of the body and that the left aorta of the embryo had dropped out between the seventh segmental artery and the junction of right and left embryonic aortae. The key to the derivatives of the left arches is that the left recurrent laryngeal nerve looped around the subclavian artery (in a normal embryo, of course, it loops around the sixth arch). Using this key, the following observations were made: the first branch of the aorta distal to the coronary arteries (an anomalous left innominate artery) was the persisting root of the right ventral aorta; the common carotid artery was the remaining third arch; the segment marked by asterisk was the retained fourth arch plus a portion of the left dorsal aorta (proximal to sixth arch); and, that segment of the subclavian artery around which the nerve looped was a persisting segment of the left dorsal aorta (between sixth arch and seventh segmental artery of the embryo).

Regarding the pulmonary circulation, the lungs of Twin I must have received less blood from the pulmonary trunk than from the aorta because the lumen of this trunk was almost occluded. Aortic blood could reach the right lung via the right sixth arch ("Duct. art."). It could reach the left lung by passing successively through the vessel marked by asterisk, the left sixth arch and the left pulmonary artery.⁶

⁵ The incidence of anomalous right subclavian artery (retroesophageal) is reported to be about 1:100 (McDonald and Anson, '40). The literature is brought up to 1941 by Hammer and Meis who report three cases of their own in which the right inferior laryngeal nerve failed to loop around the anomalous artery. As pointed out by Cairney ('25), it is difficult to arrive at an embryological explanation for those atypical cases in which the anomalous artery is said to pass between trachea and esophagus (Bradley, 1871) or in front of the trachea (Geddes, '11).

The reverse condition occurs. It may be recalled (footnote 1) that Röver's case of dicephalus tribrachius tripus exhibited an anomalous left subclavian artery which came off the anomalous right aorta and passed behind the esophagus. In patients an anomalous left subclavian artery (retroesophageal), associated with an anomalous right aorta, is said to cause difficulty in swallowing, the syndrome being known as dysphagia ludoria (Renander, '26).

⁶ Since Twins I and II died soon after birth there was no opportunity to test what effect the mixing of blood in the common atrium would have on sleep. Miller ('42), in considering a case of conjoined infants (dicephalus tribrachius dipus) whose sleep was not always synchronous, has questioned the humoral theory of sleep. The original description of the case was by Black ('40). At autopsy Black noted that the hearts were separate and that there were many anastomosing vessels.

3. *Abdominal viscera*

When the single abdominal cavity was opened, there could be seen a partially-duplicated digestive tube, a diverticulum of Meckel, two livers conjoined, a spleen belonging to Twin II and a single umbilical vein (figs. 4 and 6). The digestive tube and its dorsal mesentery were Y-shaped structures, being double above Meckel's diverticulum and single below it.

When sections of the diverticulum of Meckel were studied microscopically, it was found that intestinal epithelium lined the anomalous sac. Such aberrant structures as gastric glands and pancreatic acini (Trimingham, '43) were not observed.⁷

Dissection of the abdominal region showed that the stomach, duodenum and pancreas of Twin II were essentially normal as were also the greater omentum and the omental bursa. Although the duodenum and pancreas were not buried by the mesocolon, the mesoduodenum had fused with the abdominal wall.

In Twin I, the fundus of the stomach lay on the left side of the vertebral column and presented an abnormal appearance (bent and wrinkled), while the pylorus and duodenum passed abnormally to the left. The pancreas was C-shaped, its head being anterior to the duodenum and its tail posterior to it. The greater omentum was rudimentary and the spleen and omental bursa were absent. The mesoduodenum was free being neither fused with the abdominal wall nor covered by the mesocolon. It was widened by the pancreas which it enclosed. The lesser omentum passed upward from stomach and duodenum to the left where it attached to the liver of Twin I and to the hepatic portion of the hepato-duodenal ligament of Twin II.

Although the livers were united in the same plane as the twins, the gall bladders lay side by side in such manner that they were almost perfect mirror images of each other. The gall bladders communicated with the livers and the duodena by means of the usual biliary ducts. The common bile duct of Twin I passed in front of Duodenum I and then emptied in its anterior wall (not illustrated in fig. 6), thus showing that Duodenum I was not a mirror image of Duodenum II. In fact, when the liver was removed and the duodenum and attached structures were thrown to the right, the new pattern of asymmetry thus produced simu-

⁷ It is of interest to note in Boyden and Levin's monograph ('40) that in the Babylonian Talmud reference is made to conjoined abortuses and also to two congenital anomalies which we have observed in Twins I and II, namely agenesis of the spleen and presence of the diverticulum ilei (Meckel's). The reference to the diverticulum "anticipates by six centuries the first report of it in scientific literature."

lated the normal. This showed that the mirror imaging exhibited by Gall Bladder I was incidental and not part of a basic pattern of situs inversus viscerum.

When the conjoined livers were dissected, it was found that the two portal veins anastomosed with each other and that each communicated with the umbilical vein before branching to form afferent hepatic veins. The several efferent hepatic veins opened into the two inferior venae cavae. Finally, the ductus venosus and the inferior venae cavae united to form that "common inferior vena cava" which perforated the conjoined diaphragms (fig. 6).

Beginning about 5 cm. below the two duodeno-jejunal junctions and ending at Meckel's diverticulum, the small intestines of the twins lay side by side, looping all the while. Although they were bound together superficially by peritoneal adhesions, they were easily separated by blunt dissection which did not destroy their mesenteries. While separating them, it was observed that there were many small anastomoses between the two sets of jejunal and ileal arteries. Obviously these anastomoses could have developed only after adhesions had interrupted the peritoneum.

Distal to Meckel's diverticulum, the unduplicated portion of the digestive tube consisted of ileum, colon and rectum. The proximal third of the colon passed downwards (C, fig. 4), the middle third looped to the right and the distal third passed obliquely downward to the rectum. Although the proximal third was attached firmly to the peritoneum which covered the left kidney of Twin II, the remainder was suspended by its mesocolon. The one and only loop of colon lay in a special peritoneal fossa the orifice of which faced superiorly (this fossa also contained the rudimentary uterus and ovaries which will be described). The inferior mesenteric artery of Twin II supplied the colon and the rectum.⁸ In Twin I, no trace of an inferior mesenteric artery could be found. Considering all these observations, the abnormal position of the colon should be interpreted as having resulted from incomplete rotation of the umbilical loop of the embryonic gut and not from reversal of rotation (not mirror imaging).

4. Pelvic viscera and perineum

When the lumbar and pelvic regions were dissected, it was found that there were two sets of uropoietic and genital structures (fig. 6). While

⁸ While there may have been ileocolic and middle colic branches of the superior mesenteric arteries, their presence was not proven by dissection.

one set was essentially normal in appearance, the other set was rudimentary.

The more normal set of structures had derived its embryonic primordia from the right side of Twin I and the left side of Twin II and had terminated in the vulva. In general, the uropoietic organs resembled those of the newborn. While the lobulated kidneys had molded their suprarenal glands in the usual manner, the left kidney of Twin II had failed to complete its normal rotation. The hilum of this kidney was abnormally large. The bladder and urachus were flanked as usual by two umbilical arteries. The bladder received only five arteries, three from Umbilical Artery I and two from Umbilical Artery II. The ureters, the trigone of the bladder, the urethra and the urethral orifice looked much like those of a normal infant. Similarly, the reproductive organs resembled those of the newborn. The uterus and the vagina exhibited normal, prenatal hypertrophy (Scammon, '23), this being attributable to the stimulating action of the estrogen of pregnancy (Fraenkel and Papanicolaou, '38). The round ligaments of the uterus terminated in the major lips of the vulva. The uterine arteries arose from the umbilical arteries and passed around the ureters in the normal manner. The right ovary was supplied by the right sex artery of Twin I. While it is reasonable to suppose that the left ovarian artery was contributed by Twin II, no direct observation concerning this point could be made because the proximal segment of the artery in question had been accidentally destroyed during dissection.

The rudimentary set of urinary and genital structures had been developed from the left side of Twin I and the right side of Twin II. Only the urethra (of all the urogenital passages) obtained an outlet — and this on the posterior aspect of the specimen. The kidneys were abnormal and cystic and they lay in the lumbar region slightly above the conjoined ilia. Neither kidney contacted its suprarenal gland. The right ureter of Twin II was flattest and widest. The bladder was a retroperitoneal, pelvic organ and its shape resembled that of a thin, longitudinal slice of a pear. At the superior tip of the bladder, no trace of a urachus could be found. Lateral to and slightly below this point, the flattened lumen of the bladder received the orifices of the ureters. Inferiorly, the lumen became narrow and merged with that of the urethra which in turn, as already mentioned, terminated on the posterior aspect of the specimen. The bladder received a pair of arteries from each of the attenuated post-iliac aortae (e.g., median sacral arteries) but none from any other source. In regard to the genital structures, the utero-vaginal canal failed to reach the perineal region. Instead, it became occluded near its

distal end; the solid cord, thus produced, terminated by blending with the apical wall of the bladder. This was confirmed microscopically by observing stained serial sections of a block of tissue which included adjacent portions of uterus, ureters and bladder. Incidentally, it was found that in the subterminal region of the uterus there were many uterine glands.

Dissection of the perineal region showed that the single pelvic outlet was closed in part by two diaphragms each of which was composed of layers from the right side of Twin I and the left side of Twin II. The urogenital diaphragm was perforated by the vagina and the more normal urethra; this diaphragm presented a normal appearance. However, the pelvic diaphragm was incomplete. It consisted only of symmetrical halves which by failing to unite left a v-shaped gap for the passage of the urethra, the vagina and the rectum. This gap was narrowest between the pubic rami and widest between the coccyges. Because of this gap, all of the pelvic viscera sagged downwards causing the perineal region to bulge. The flat, rudimentary bladder received little or no support from the pelvic diaphragm. In fact, the lower half of it lay below the diaphragm. If traces of a second set of diaphragms were present, they were so small that they could not be identified.

Regarding the embryology of the pelvic viscera, the non-duplication of that portion of the digestive tube which originated from the embryonic hindgut (portion extending from Meckel's diverticulum to anus) suggests that only one embryonic cloaca had been formed. It is clear that four wolffian ducts had entered the cloaca because four kidneys had developed. Also, four müllerian ducts (cf. the four uterine tubes) must have reached the cloaca and have become aligned in such manner that two utero-vaginal canals were formed; of these canals, only one was normal enough to furnish a primordium of the upper segment of a vagina. Presumably the müllerian ducts had been steered to the cloaca by the wolffian ducts — Gruenwald ('37) having reported that in chick embryos the experimental destruction of the caudal end of the wolffian duct prevents the müllerian duct from reaching its normal destination.⁹ The presence of two urinary bladders shows that two urogenital sinuses had been partitioned off the cloaca. The planes of partitioning must have been such that each twin contributed two tubes to each sinus, namely one wolffian duct and one müllerian duct. The flat, rudimentary bladder originated from one urogenital sinus (vagina

⁹ In a more recent paper, Gruenwald ('41) reports that in normal human embryos the growing tip of the müllerian duct is situated within the basement membrane of the wolffian duct where it may receive cells from the latter duct.

absent). The more normal bladder, the distal segment of the vagina (Koff, '33) and all (?) of the vaginal epithelium (Meyer, '37; Burns, '42) originated from the other urogenital sinus.

The exact nature of the cloacal membrane can only be surmised. However, its three subdivisions — one anal and two urogenital membranes — must have been arranged in a row, the anal membrane being in the middle (between the other two but to one side of the midline of the specimen). This arrangement is unlike that observed in embryos of a special strain of mice in which about 20% of the young exhibit duplication of the caudal region of the body (Danforth, '30).

Although it is possible to visualize how that urogenital sinus from which the more normal bladder developed could have been split off, it is not clear as to how the second urogenital sinus could have been formed. Undoubtedly the problem could have been solved if a satisfactory model existed of the 13-mm. stage of *iliothoracopagus* which was described by Viana ('22).¹⁰

DISCUSSION

Wilder ('08) concludes that in the development of identical twins that are symmetrically conjoined (*cosmobia*), there is "neither a 'fusion' of parts already formed nor a gradual development from the normal towards the abnormal during embryonic life, but the parts appear doubled or reduced from their first appearance and their development is controlled in the same way as are the bilateral structure and other architectural characteristics of normal beings." While this is a useful conception and means, when couched in modern terms, that the development of contiguous embryonic axes, wherever they are sufficiently

¹⁰ Viana did not present a model of the specimen. His illustrations of sections through the pelvis show one rectum, one allantois, four ureters and three of the four wolffian ducts. While his diagram (fig. A) shows two wolffian ducts entering a urogenital sinus (erroneously labeled "allantois"), it fails to show where third and fourth wolffian ducts emptied. In describing the specimen, he failed to mention whether or not there was a rudimentary (second) urogenital sinus. That it must have been present, unless it had undergone retrogression, is indicated by the presence of third and fourth ureters and of their corresponding, developing metanephroi. Boyden ('32) has shown that in man when the wolffian duct fails to reach the cloaca, agenesis of the kidney ensues; and he observed that the metanephric blastema fails to differentiate into secretory tubules when there is non-union of metanephric bud (inductor) and blastema. Gruenwald's observations ('39) are confirmatory. Presumably the inductive action of the ureteric bud is more or less specific because Gruenwald ('43) has reported that when the metanephric blastema of chick embryos is deprived of its normal inductor (ureteric bud) by grafting nervous tissue in the path of the growing wolffian duct, the grafted tissue acts as inductor but causes this blastema to form only tubules which resemble those of the mesonephros. (I am indebted to Dr. Elizabeth Nissen, Assistant Professor of Romance Languages, for translating a portion of Viana's paper.)

close to act as one, is subject to the same principles of induction as that of a single axis, it is quite conceivable that structures produced in accord with these forces could still become blended by subsequent extension or by the breakdown of intervening tissue. Thus paired hearts might induce paired hepatic outgrowths which upon entering a common tissue could unite to form a single liver, or adjacent walls of thin-walled contiguous atria might be absorbed to form a common atrium in the same way that right and left endocardial tubes unite to form a single chamber in the normal embryo.¹¹ In these instances and to the extent noted, therefore, Twins I and II require some modification of Wilder's rule.

A related but different problem is the manner of origin of identical twins.¹² At first it was thought that the dissection of Twins I and II might assist in determining whether the "fission" theory of Newman ('23) is more acceptable than the "budding" theory of Stockard ('21). The "fission" theory maintains that identical twins originate by separation of right and left halves of the developing embryo (ovum) and that incomplete separation results in the formation of cosmobia. As support for his theory Newman cites those cosmobia in which the right twin exhibits mirror imaging of the viscera (*situs inversus viscerum*).¹³ As examples of "incomplete symmetry reversal" he mentions such anomalies as right aorta. In order to explain those cases in which the right twin fails to show mirror imaging, he assumes that the separation had

¹¹ In experiments in which chick blastoderms were transplanted to the chorio-allantoic membrane, Willier and Rawles ('31) noted that liver did not develop in the absence of heart.

¹² Although it is generally agreed that in man identical twinning is probably due to a temporary slowing of metabolism of the ovum at a critical moment during development (during or near the period of gastrulation), it is thought that identical twins may arise by any one of the following methods: (1) division of the blastoderm before the primitive axis has been laid down, (2) de-axiation of the ovum so that double gastrulation occurs and (3) separation of right and left halves of the "single embryonic axis" (Newman, '23; Arey, '42). Opposing theories formulated to explain the origin of conjoined as well as separate twins are the "budding" theory (Stockard, '21) and the "fission" theory (Newman, '23). The essence of the "budding" theory is that if the growth of the primary embryonic axis becomes arrested "the usual single axis does not arise with sufficient influence to suppress the origin of other axes, and twins or double axes result" and that "the degree of duplicity in double individuals depends upon the distance apart of the embryonic buds on the blastoderm."

As to what extrinsic factor might have induced the formation of Twins I and II, the clinical report provides no clue — the mother could recall no untoward event during the time of expected gastrulation (see footnote 1). Concerning this time, it is known from observations on the youngest human embryo thus far discovered that by $7\frac{1}{2}$ days after conception the process of gastrulation has already begun (Hertig and Rock, '44).

¹³ It is reported that in man the incidence of *situs inversus viscerum* is at least 1:5000 (Chudnoff and Shapiro, '39).

been complete enough to permit the development of normal asymmetry ("situs solitus"); however, he maintains that in the right twin "situs solitus" represents a "real symmetry reversal, for the right-hand component is expected to be a mirror image of the left, and should show right-hand asymmetry (situs inversus) of asymmetrical structures" (p. 174).

Although reflection on the possible origin of Twins I and II soon led to the realization that dissection of cosmobia is not a fruitful approach to the question as to which of the opposing theories is the more acceptable, it still should be pointed out that in Twin I there were no manifestations of situs inversus viscerum which might have been caused by reversal of such normally dominant processes as rotation of the stomach to the left or dextral torsion of the heart.¹⁴ Thus the abnormal rotation of the duodenum occurred in spite of the fact that the embryonic stomach had rotated to the left as in a normal embryo, the evidence being that the fundus of Stomach I lay on the left side of the vertebral column. Also the right fourth aortic arch persisted in spite of the fact that the heart had looped to the right as in a normal embryo, the evidence for this fact being the final topographical relations of right and left ventricles and of the vessels which passed from them. It is concluded that from the standpoint of embryology there are no grounds for assuming, with Newman, that such anomalies as right aorta are predetermined by the origin of the right twin from the right half of the developing embryo. Presumably such "predetermination" could not transpose the aorta without first reversing dextral torsion of the heart.

¹⁴ There are records of at least five human cosmobia in which the right twin exhibits situs inversus viscerum which might have been caused by reversal of such processes (cases 50, 60, 71 and 102 reported by Fisher, 1866 and the case described by Morrill, '19). In conjoined twins occurring in salmon and trout, such cases have been observed (Komai, '38; Morrill, '19; Newman, '23, '31a, '31b, '40a, '40b). In respect to chick embryos Waddington ('41) questions the theory that conjoined chick embryos originated by "fission of the embryonic axis" (Newman, '40b).

Incidentally, Morrill's statement ('19) concerning Benedini's case of dicephalus tribrachius tripus (1844) should be corrected. Morrill states that "the gall bladder, stomach, pancreas, spleen and intestines were all single." He takes this from Fisher (1866) but he must have overlooked Fisher's other statement that "the viscera of the little abdomen was very rudimentary, the intestines were small, and empty, except the ilium [ileum], in which meconium was found, and which terminated in the cecum of the anterior cavity." In any event, an English abstract of Benedini's Italian paper (1844) makes it clear that "in the posterior abdomen there was a [another] small liver" and that "the small intestines were double, and the large single."

The question then arises as to whether the left twin could induce the development of mirror imaging in the right twin.¹⁵ The experiments cited by Child ('41) would seem to favor such an hypothesis, for when certain organisms (hydranths of *Corymorpha*) or portions of an embryo (amphibian limb-buds) develop side by side, with cellular continuity, mirror imaging is the general rule. In regard to the mirror imaging of conjoined appendages in *Amphibia*, Child points out that if it is due to a physiological differential, it may be interpreted as follows: "the high or low side of the differential is common to both, or the differential in one may induce a differential symmetrical to it in the other."

Pending the gradual accumulation of experiments on mirror imaging or the ultimate discovery of embryonic stages of identical twins that are younger than those now on record (Streeter, '19; Arey, '22; Viana, '22; Ysander, '24), it would seem that both theories should be viewed as useful working hypotheses.¹⁶

CONCLUSIONS

A detailed dissection of conjoined fetuses of the seventh lunar month has been presented with a view to determining what bearing such information would have on the current theories of twinning. Apparently Wilder's rule concerning the development of twins that are symmetrically conjoined (*cosmobia*) should be somewhat modified. Contrary to Newman, the occurrence of such anomalies as right aorta in the right member of *cosmobia* does not constitute evidence which supports the "fission" theory. It is an open question as to whether the development of *situs inversus viscerum* in the right twin is caused by the left twin acting as inductor. At present the "budding" and "fission" theories should be considered as useful working hypotheses.

¹⁵ It would seem that mirror imaging of the finger prints of separate identical twins (Newman, '40a) militates against that interpretation of "*situs solitus*" which Newman presented in 1923 (see Discussion, last sentence of second paragraph). On the other hand, Newman's view ('40a) in regard to the significance of mirror imaging of the finger prints has been brought into question by more recent observations. In the words of Rife and Cummins ('43), "Data assembled by three independent investigators and analyzed by different individuals agree in revealing that "mirror-imaging" in dermatoglyphics occurs no more frequently in monozygotic twins than in random pairs of unrelated individuals, sibs and fraternal twins."

¹⁶ Before closing our discussion of theories of twinning, an erroneous statement by Krafka ('42, p. 263) should be corrected. He says, "Two observations by Blasius ('39) on *thoracopagii*, add a confusing factor to the problem [sex of identical twins], since one member of each set of conjoined twins was male while the other was female." Actually, Blasius reports that in case I the right twin was a female and the left twin a female pseudo-hermaphrodite, and that in case II the twins were females (beide Mädchen).

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PLATE 1

EXPLANATION OF FIGURES

All photographs on this plate were made by Mr. Henry Morris, Medical Photographer.

1 Photograph of Twins I and II ($\frac{1}{3}$ actual size). The larger thoracic and abdominal walls face the observer, as does also the clitoris (not visible).

2 Another photograph of the twins ($\frac{1}{3}$ actual size). The smaller thoracic and abdominal walls face the observer. The third upper appendage is below Right Ear II and Left Ear I. The third inferior appendage hides the second urethral orifice.

3 Photograph of a roentgenogram of the twins. Although the adjacent ilia were united by cartilage, this feature cannot be made out in the photograph. For additional explanation, see text.

4 Photograph of certain abdominal structures that were revealed when the abdominal cavity was opened ($\frac{2}{3}$ actual size). Specimen oriented as in figure 1. C, colon; II. I, Ileum I (it hides the corresponding portion of Ileum II); I.-c. j., ileocecal junction; Jej. I, Jejunum I; Jej. II, Jejunum II; M.d., Meckel's diverticulum; U. il., unduplicated portion of the ileum; U.v., umbilical vein; V.p., vermiform process (its free end is indicated by the guide line).

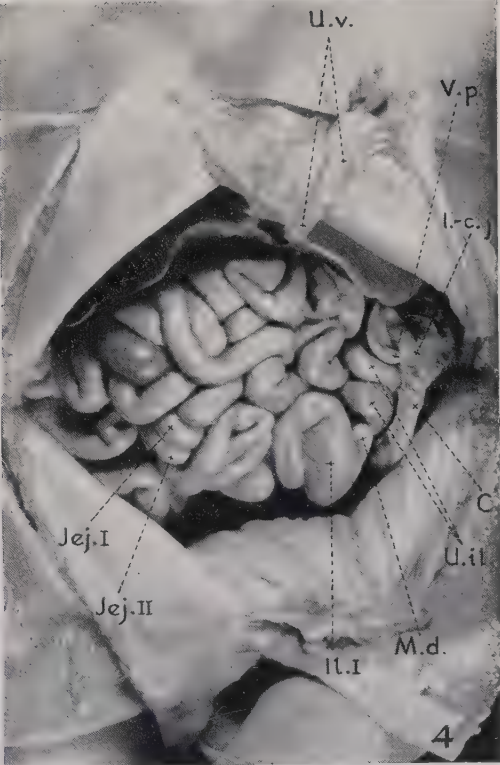
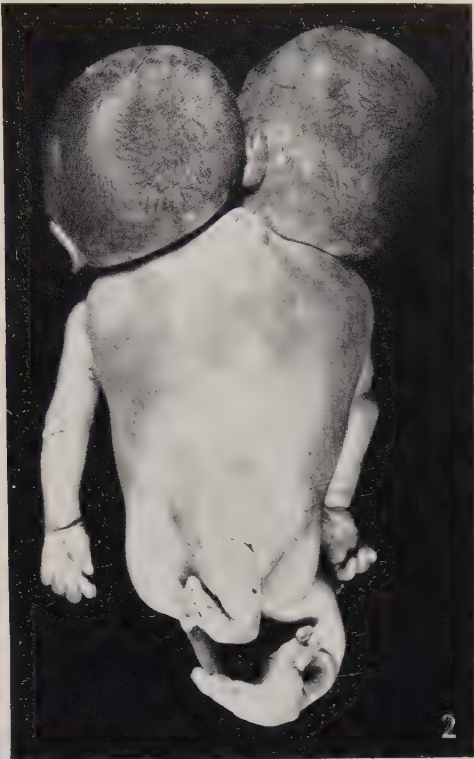
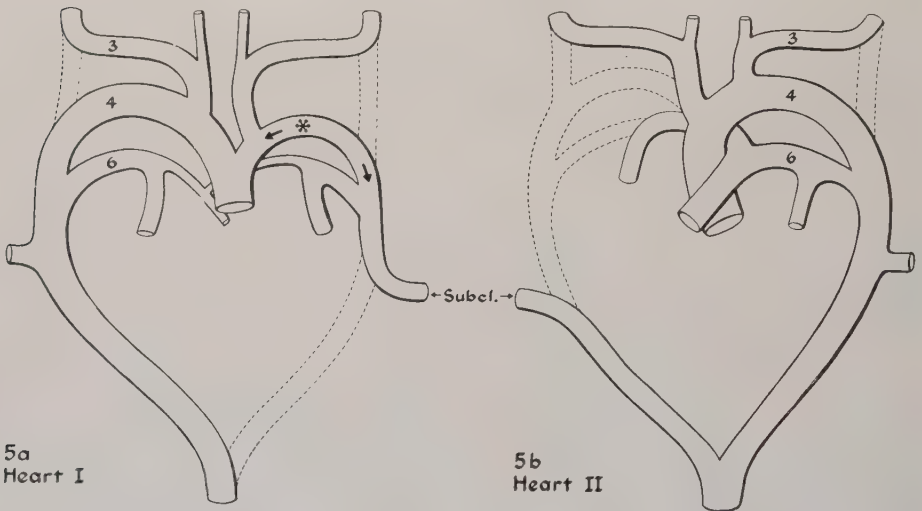


PLATE 2

EXPLANATION OF FIGURES

5 Drawing of a dissection of the conjoined hearts and certain adjacent thoracic structures ($\times 1\frac{3}{4}$). The orientation is the same as that in figure 1, i.e., with the heart of the right twin (I) to the reader's left. In Heart I, the asterisk marks the persisting left fourth aortic arch and a persisting segment of the left dorsal aorta of the embryo (that portion of it proximal to the sixth arch). In Heart II the dash lines indicate the anomalous path of the right subelavian artery and its point of origin from the aorta.



5a and 5b Diagrams suggesting the method by which the arteries of each heart were derived from the embryonic pattern.

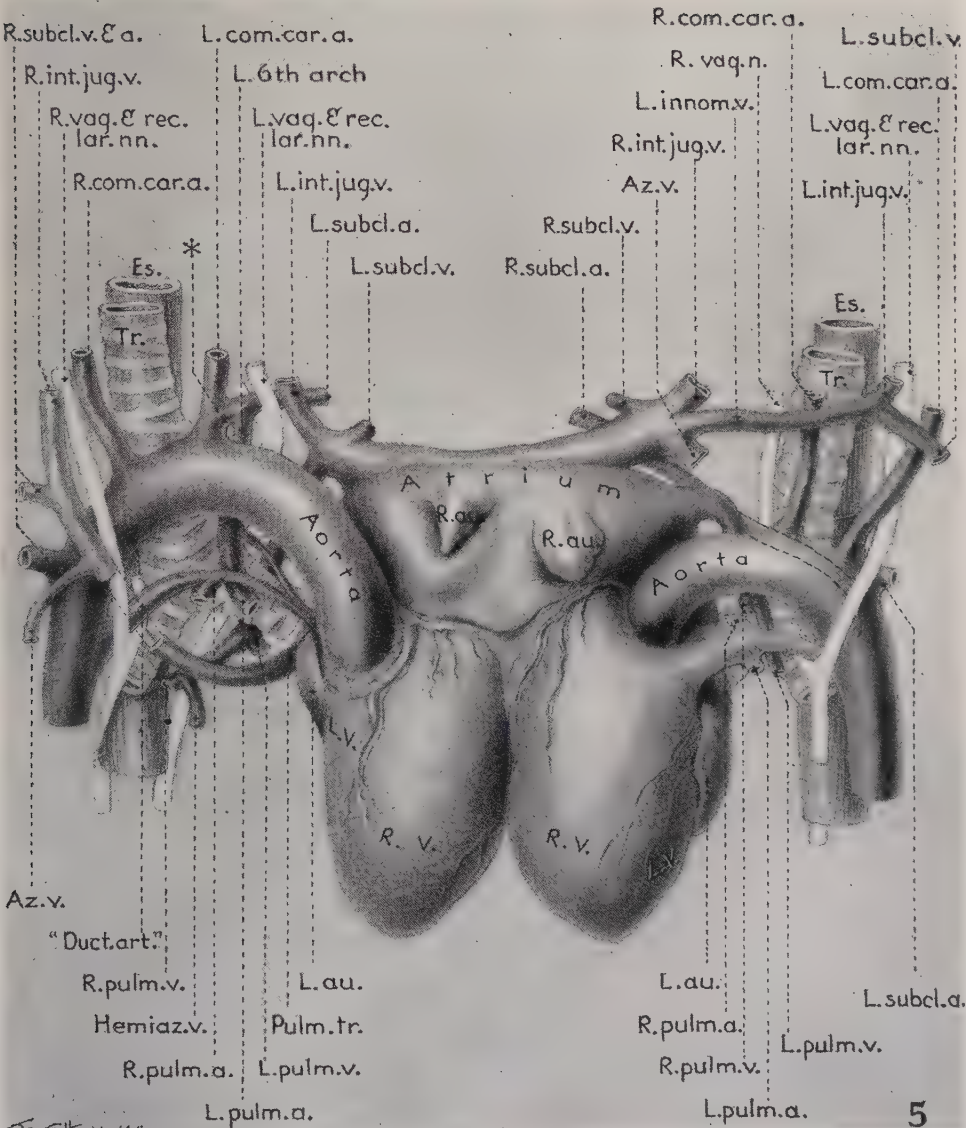
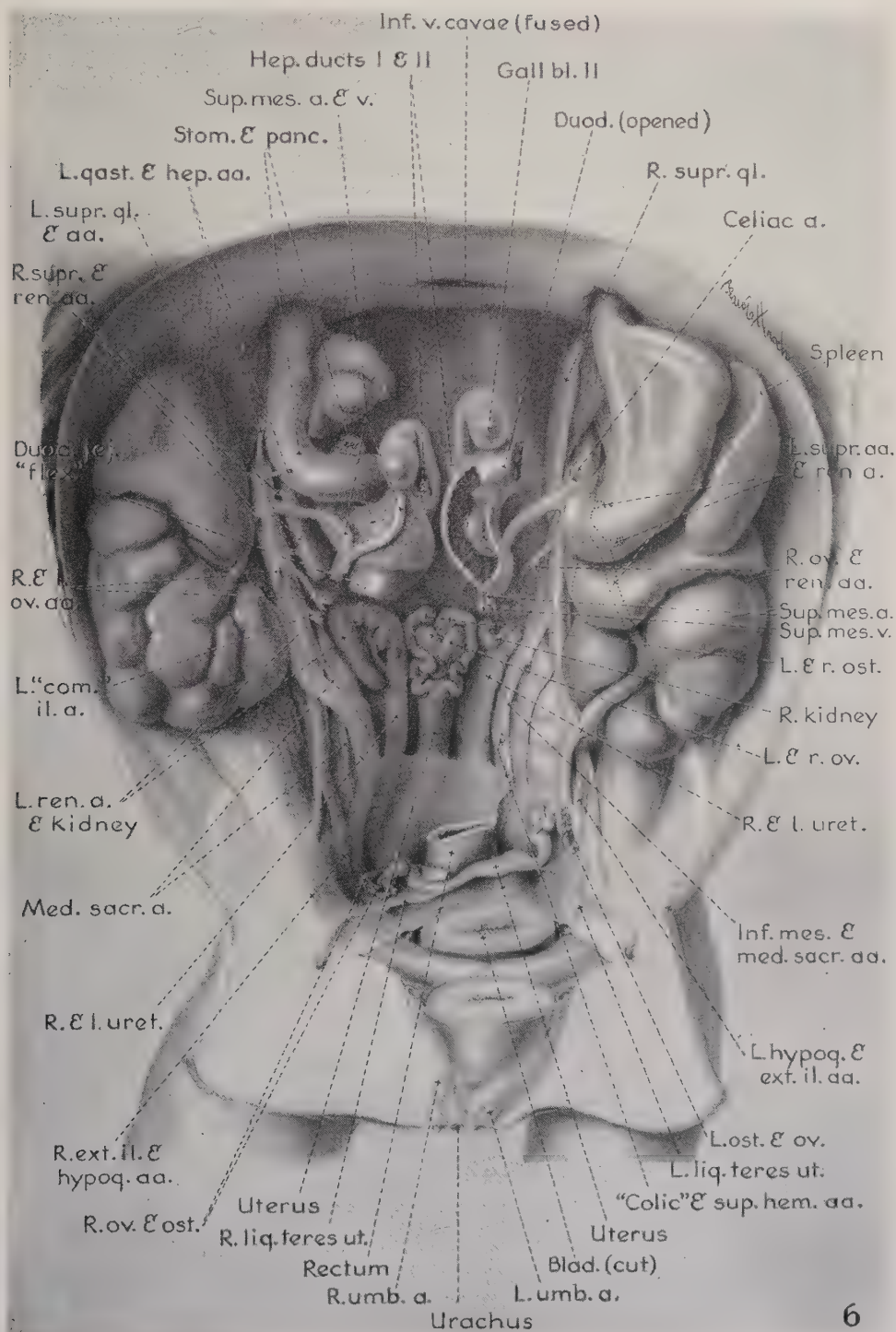


PLATE 3

EXPLANATION OF FIGURES

6 Drawing of a dissection of certain abdominal and pelvic structures ($\times 1\frac{1}{4}$). In each twin the inferior vena cava (not illustrated) lay on the right side of the abdominal aorta. The two inferior venae cavae united to produce the "fused inferior venae cavae" which perforated the conjoined diaphragms. The peritoneal relations of the rudimentary set of genital structures were so complicated that it was decided not to try to illustrate them. While the drawing gives the false impression that the rudimentary genital structures were retroperitoneal, they lay in that special peritoneal pouch which also contained the colic loop (see text) — it should be stated that: (1) the upper portion of the uterus was surrounded by peritoneum, (2) the peritoneum reflected off the uterus (in front and behind) at a point slightly above that cut edge of peritoneum illustrated, thereby leaving the lower segment of this organ without an intrinsic peritoneal covering and (3) blood vessels reached the uterus, the uterine tubes and the conjoined ovaries by passing through a rudimentary broad ligament. The fundus of Stomach I (wrinkled) lay on the left side of Vertebral Column I. Inside Duodenum I, the major and minor papillae opened to the observer's left — thus showing that Duodenum I was not a mirror image of Duodenum II, as did also the fact that Common Bile Duct I passed in front of Duodenum I (through substance of Pancreas I).



NEUROSECRETION

VIII. THE NISSL SUBSTANCE IN SECRETING NERVE CELLS

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TWO TEXT FIGURES AND ONE PLATE (TEN FIGURES)

In a recent review of neurosecretion the hypothesis has been advanced that the products of the neurosecretory process originate in association with the basophil constituents of the nerve cells (Scharrer and Scharrer, '45). The evidence concerning the relation of secretory granules to one of these basophil substances, namely, nuclear chromatin, has already been discussed in some detail (Palay, '43). The concept will be further documented in this paper by a study of the Nissl substance in secreting nerve cells.

MATERIAL AND METHODS

The cells investigated here are those of the nucleus preopticus and nucleus lateralis tuberis in the teleost fishes *Ameiurus nebulosus*, *Noturus flavus*,² *Fundulus heteroclitus*, *Centropomus striatus*, and *Tautoga onitis*;³ of the nucleus preopticus in the amphibians *Bufo terrestris* and *Bufo americana*; of the nuclei supraopticus and paraventricularis of the banded water snake, *Lapemis hardwickii*,⁴ and of the dog.⁵ The standard method for the study of the relations between secretory material and Nissl substance was as follows: Whenever possible a cannula was inserted into the heart of the anesthetized animal, and the blood was washed out with plain Zenker's solution. Next Zenker-formol was injected. Perfusion of the brain with the fixing fluid was in this way maintained for periods up to 15 minutes. Then the brain was removed and left in Zenker-formol for 6 to 12 hours, after which time it was washed in running water for 24 hours. The material was embedded

¹ This study was aided by a grant to Western Reserve University from the Rockefeller Foundation.

² These fishes were collected at the F. T. Stone Laboratory at Put-In-Bay, Ohio.

³ These species were obtained at the Marine Biological Laboratory, Woods Hole, Mass.

⁴ The specimens were collected at Manila, P. I., in 1938.

⁵ A number of dogs were obtained from the Cornell farm through the kindness of the late Dr. C. R. Stockard.

in celloidin (nitrocellulose), or in paraffin over alcohol, methylbenzoate, and benzene. Celloidin and paraffin sections were stained with Foot's modification of Masson's trichrome stain. Special methods and additional material were used for specific purposes. Reference to these will be made in the text.

THE NISSL PATTERN OF SECRETING HYPOTHALAMIC NUCLEI

The nuclei preopticus and lateralis tuberis of fishes and the nucleus preopticus of amphibians are composed of cells which differ from other nerve cells in the brain in that the Nissl bodies are characteristically aggregated in the periphery of the cell body (figs. 1 A-F). The cells of the homologous nuclei supraopticus and paraventricularis in reptiles show the same Nissl pattern (fig. 1G). In mammals this arrangement of the Nissl bodies has been observed by previous investigators. Thus

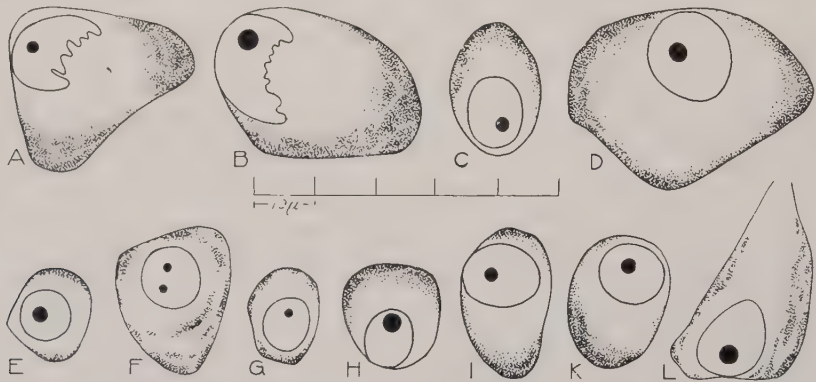


Fig. 1 Nissl patterns. A. Nucleus preopticus of tautog (*Tautoga onitis*). B. Nucleus preopticus of puffer (*Spheroideus maculatus*). C. Nucleus preopticus of killifish (*Fundulus heteroclitus*). D. Nucleus preopticus of eel (*Anguilla rostrata*). E. Nucleus preopticus of catfish (*Ameiurus nebulosus*). F. Nucleus preopticus of bullfrog (*Rana catesbeiana*). G. Nucleus paraventricularis of banded water snake (*Lapemis hardwickii*). H. Nucleus supraopticus of rat. I. Nucleus supraopticus of rabbit. K. Nucleus supraopticus of dog. L. Nucleus supraopticus of man.

Ingram ('40, p. 201) describes the "Nissl substance heavily condensed at the periphery of the perikaryon" leaving the "immediate perinuclear area strikingly free of Nissl material" in the supraoptic and paraventricular nuclei of the primate hypothalamus. Our own observations confirm this description for the cells of the supraoptic and paraventricular nuclei of the rhesus monkey, sheep, goat, dog, cat, guinea pig, rat, and opossum (figs. 1 H-K).

The pattern is constant under various conditions of fixation. It does not depend upon the choice of the fixing fluid. Thus, the Nissl substance

occupies the periphery of the cells after fixation in alcohol, Bouin's fluid, Regaud's fixative, Zenker-formol, or 10% formalin. As mentioned above, in most of our material rapid fixation by perfusion through the vascular system was employed. But the characteristic Nissl pattern does not depend upon this special procedure. Even when post mortem changes are unavoidable, as in human material (fig. 1L), the Nissl bodies show the same arrangement (Greving, '28, p. 998).

CHANGES IN THE NISSL SUBSTANCE DURING THE SECRETORY PROCESS

In the secreting nerve cells under consideration a spatial relationship is observed between the Nissl substance and the smallest acidophil granules. The Nissl bodies are interspersed with the secretory granules in the peripheral zone of the cell body (figs. 4 and 6). Evidently, however, in the process of accumulation of granules the relationship does not remain merely a spatial one. Only in cells with few granules does

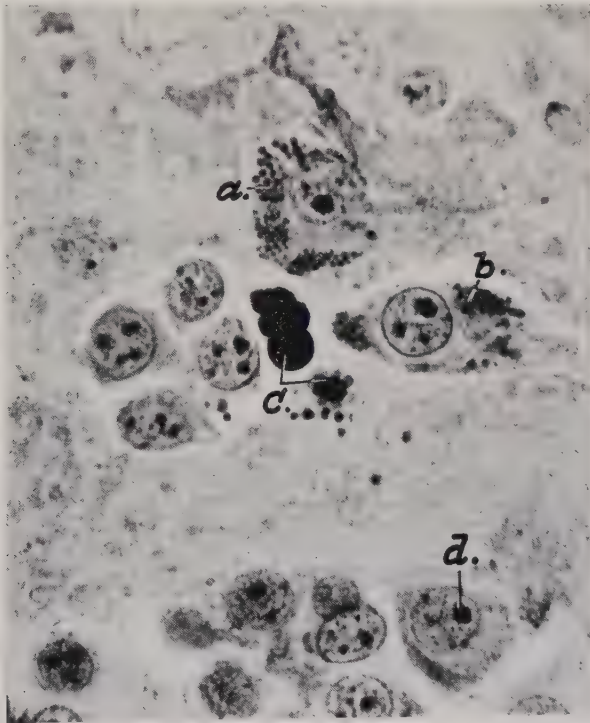


Fig. 2 Nucleus preopticus of the toad (*Bufo americana*). a, and b, cells with peripherally located secretory granules; c, cluster of discharged granules and droplets; d, cell with peripherally located Nissl bodies, but without acidophil granules. See also figure 6. Zenker-formol, nitrocellulose, 7μ , Mallory-azan stain, photomicrograph, $1000\times$. (From E. and B. Scharrer, Res. Publ. Ass. nerv. ment. Dis., vol. 20, fig. 83, p. 181, 1940).

the amount of Nissl substance appear unchanged, whereas in cells with numerous granules proportionately less Nissl substance is found. Thus the granules do not simply displace the Nissl bodies in the peripheral region of the cell, but they actually replace them.

This inverse relationship between the amounts of Nissl substance and of neurosecretory material becomes obvious from a comparison of the cells illustrated in figures 3 to 5. The cell of figure 3 shows in a catfish the typical Nissl pattern described in the preceding chapter. In another cell (fig. 4) a few small acidophil granules appear within the peripheral Nissl substance. In a third cell (fig. 5) which was found lying next to that of figure 3, practically no Nissl substance is evident, and the periphery is almost exclusively occupied by the acidophil granules. These are disposed in the same pattern as the Nissl substance in the cell illustrated in figure 3. Another example of an intermediate stage in this process as observed in the sea bass, *Centropristes striatus*, is shown in figure 7. Such cases of partial transformation of the Nissl region into neurosecretory material are common in fishes and toads; the complete disappearance of the Nissl substance and its replacement by acidophil granules as shown in figure 5 is comparatively rare.

The Nissl substance is similarly involved in cases where the neurosecretory process manifests itself in ways other than the production of granules. Thus, in the teleosts *Tautoga* and *Fundulus*, vacuoles develop in the periphery of the cell which may replace so much of the Nissl substance that only a thin basophil border separates the vacuoles from the perinuclear cytoplasm. These vacuoles are filled with a homogeneous substance that stains green in Masson preparations (fig. 8). The vacuoles may take up the entire Nissl area and may contain red staining granules (fig. 9), or ghosts of granules which stain only faintly. This might indicate that acidophil granules are sometimes discharged into the vacuoles, where they dissolve.

Another variation occurs in the nuclei supraopticus and paraventricularis of the snake *Lapemis* where the peripheral Nissl region becomes increasingly transformed into a homogeneous acidophil material, a process which may finally lead to the complete disappearance of the Nissl substance (figs. 10 and 11). This type of neurosecretion is sometimes observed in fishes (fig. 12). In the course of a cytological study of the hypothalami from more than forty dogs⁶ this kind of transformation of the peripherally located Nissl substance into a homogeneous

⁶ This study has been carried out by one of us (E. S.) jointly with E. Vicari at the suggestion of the late Dr. C. R. Stockard.

acidophil material was frequently found and appears to be characteristic of the dog.

In histological preparations the actual process of elaboration of granules can, of course, not be observed. But if cells with various amounts of neurosecretory material in the peripheral cytoplasm are found, they can be arranged in series as in figures 3 to 5. It may be assumed, although it cannot be proved, that these cells represent progressive phases in the process of neurosecretion. In a sequence such as that illustrated in figures 3 to 5 the smallest granules appear first within the peripherally located Nissl substance which eventually may be completely replaced by acidophil material. This initial relationship between the small acidophil granules and the Nissl substance becomes distorted when the granules grow very numerous or coalesce to form larger drop-lets so that they fill the cell body more or less completely. In this stage little or no Nissl substance is found, and it may be concluded that it has in some way been consumed by the secretory process.

CHEMICAL RELATIONS

Attempts were made to supplement the morphological findings by the application of methods which might give some indications as to the chemical relationship between Nissl bodies and chromatin on the one hand, and the acidophil material on the other hand. The possibilities in this respect are limited by the minute quantities of the substances under investigation. The methods tried here include Feulgen's stain and the determination of the ultraviolet light absorption.

The Feulgen reaction indicates, when positive, the presence of thymonucleic acid. It is positive for nuclear chromatin and negative for Nissl bodies. Neurosecretory granules, both the intranuclear type and those associated with the Nissl substance are consistently Feulgen negative in Zenker fixed material of fishes and amphibians. If the Feulgen reaction is not masked by the presence of other substances it may be concluded that neurosecretory granules do not contain thymonucleic acid.

Through the kind cooperation of Dr. George Lavin of the Rockefeller Institute for Medical Research, New York, attempts were made to study the ultraviolet light absorption of intranuclear neurosecretory granules in *Ameiurus nebulosus* and of cytoplasmic granules in *Fundulus heteroclitus*. However, secretion granules could not be identified in the photographs obtained, although their presence was ascertained by histological methods. Consequently nothing definite can be said now concerning the ultraviolet light absorption of neurosecretory material.

DISCUSSION

A discussion of the significance of the spatial relation between Nissl substance and the acidophil material produced by secreting nerve cells must start with the question whether the Nissl picture as seen in a histological preparation reflects the state of the living cell. It has been maintained that Nissl bodies may not exist as such in the nerve cells but are artefacts resulting from the precipitation of protein substances by the fixing fluids employed in histological technique (for the literature see Bensley and Gersh, '33). This view is not supported by recent observations. Nissl patterns that are essentially the same as those observed in material fixed with the usual histological methods may be obtained by techniques in which freezing and drying is used in place of protein precipitating fluids (Bensley and Gersh, '33). Decisive evidence in favor of the existence in the living cell of Nissl bodies as discrete particles was presented by Beams and King ('35) through the application of the ultracentrifuge. In nerve cells centrifuged at high speeds the Nissl bodies move as discrete particles through the cytoplasm the viscosity of which is lower than that of the Nissl bodies. These investigators conclude from their observations that in the living cell the Nissl bodies are probably distributed as irregular composite bodies in patterns similar to those seen in histological preparations.

If the evidence is accepted that the Nissl substance is not precipitated at random by the fixing agent, but is located in the living cell according to the same pattern as in the histological preparation, the association of the secretory granules with the Nissl substance is significant. The inverse ratio of the amounts of Nissl substance and secretory material furthermore suggests that the Nissl substance diminishes to the extent to which the secretory granules increase.

In evaluating these observations one must keep in mind that they concern relatively late events in the synthesis of the secretory material. "Secretory granules have a disconcerting habit of suddenly emerging from the submicroscopic to the microscopic level without leaving adequate traces of the point of their origin" (Dawson, '42, p. 234). What happens in the submicroscopic range is not known. It is only an assumption, therefore, although a reasonable one, that an association between granules and Nissl bodies is carried over from the period of submicroscopic synthesis to the period of maturation of the granules when they become visible under the microscope. But even with the limitations imposed by the available cytological techniques it seems fairly evident that in the cases here described the Nissl bodies are affected by the

neurosecretory process. To which extent they contribute to the elaboration of acidophil material remains an open question.

SUMMARY

The relations between the Nissl substance and the acidophil material produced by secreting nerve cells were studied in representatives of fishes, amphibians, reptiles, and mammals. It was found that neurosecretory material in most cases appears to originate within the Nissl substance. The latter disappears to the extent to which the secretory product of the cells accumulates. It is concluded that the Nissl substance has something to do with the formation of acidophil material in secreting nerve cells, but the specific role of the Nissl bodies has not been determined. No light is thrown on the chemical relationships between the Nissl bodies and the acidophil granules by the techniques available at present (Feulgen reaction, ultraviolet light absorption).

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PLATE 1

EXPLANATION OF FIGURES

3 Nucleus lateralis tuberis of the catfish (*Ameiurus nebulosus*). The Nissl bodies are typically located in the periphery of the cell body. A deep invagination of the nucleus contains basophil cytoplasm. In the nucleus lies a small acidophil inclusion as described previously (Palay, '43). This cell was observed in the same section as the one illustrated in figure 5. Zenker-formol, nitrocellulose, 7μ , Masson's stain.

4 Nucleus preopticus of the eatfish (*Noturus flavus*). In the drawing two consecutive sections of the same cell have been combined. In the peripherally located Nissl substance lie numerous small acidophil granules. Zenker-formol, paraffin, 7μ , Masson's stain.

5 Nucleus lateralis tuberis of the catfish (*Ameiurus nebulosus*). The cell lies next to the one illustrated in figure 3 in the same section. The Nissl bodies have been completely replaced by acidophil granules. Zenker-formol, nitrocellulose, 7μ , Masson's stain.

6 Nucleus preopticus of the toad (*Bufo terrestris*). Acidophil granules within the peripherally arranged Nissl bodies. See also figure 2. Zenker-formol, paraffin, 7μ , Masson's stain.

7 Nucleus preopticus of the sea bass (*Centropristes striatus*). Binucleated cell with peripheral layer of neurosecretory granules. The Nissl substance is reduced to a narrow margin. Zenker-formol, paraffin, 7μ , Masson's stain.

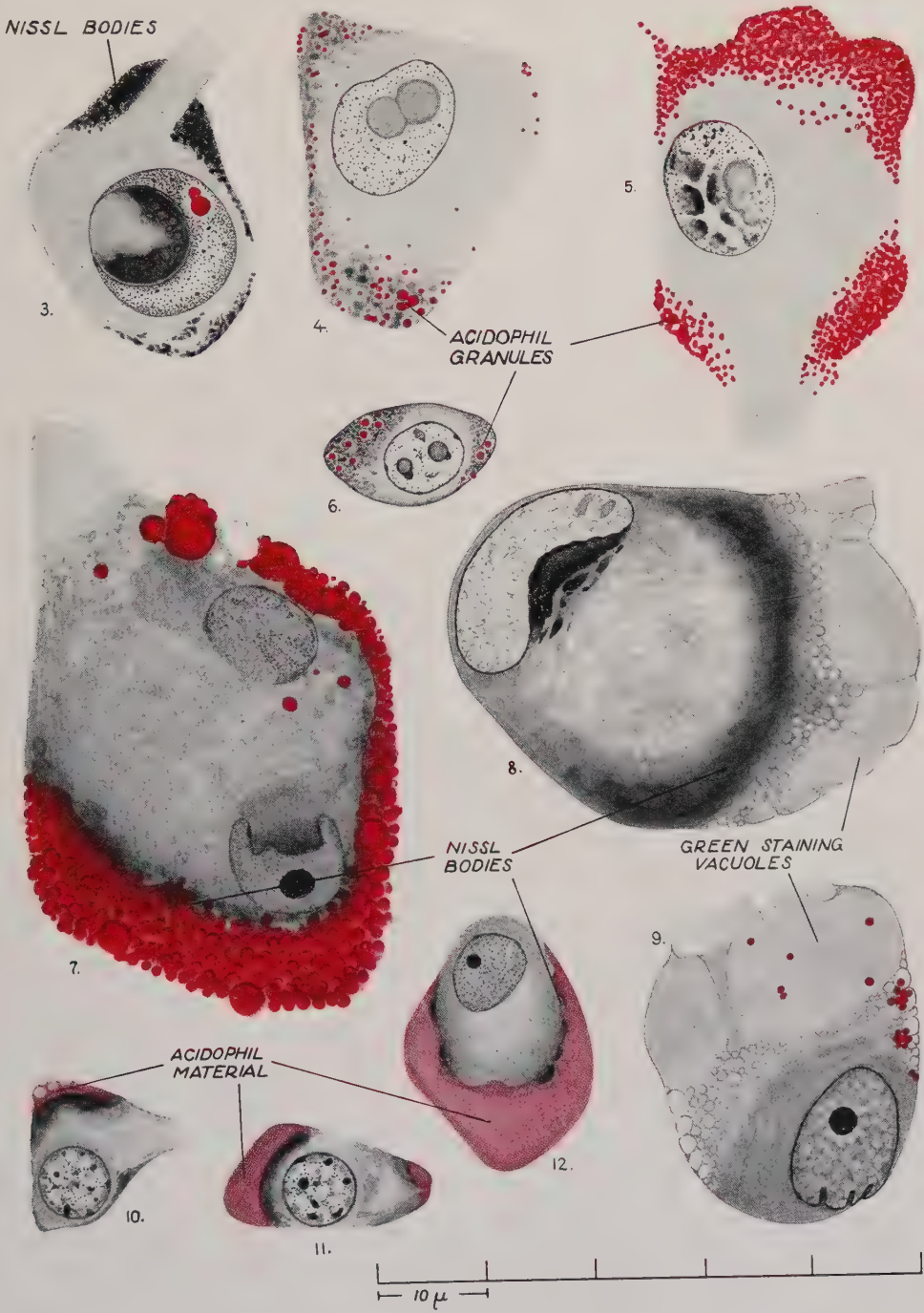
8 Nucleus preopticus of the tautog (*Tautoga onitis*). The peripherally located Nissl substance is partially vacuolated. The contents of the vacuoles stain green. The nucleus lies eccentrically; an invagination contains strongly basophil cytoplasm. Zenker-formol, paraffin, 7μ , Masson's stain.

9 Nucleus preopticus of the tautog (*Tautoga onitis*; same specimen as figure 8). The Nissl substance has disappeared. In the peripheral region are acidophil granules some of which lie scattered over the homogeneously green-staining content of a large vacuole. Zenker-formol, paraffin, 7μ , Masson's stain.

10 Nucleus paraventricularis of the banded water snake (*Lapemis hardwickii*). The margin of the Nissl area stains light red and is vacuolated. Bouin's fluid, nitrocellulose, 15μ , Masson's stain.

11 Nucleus paraventricularis of the banded water snake (*Lapemis hardwickii*). The transformation of the Nissl substance into homogeneous acidophil material is more extensive in this cell. Bouin's fluid, nitrocellulose, 15μ , Masson's stain.

12 Nucleus preopticus of the catfish (*Noturus flavus*). Transformation of Nissl substance into acidophil material. Zenker-formol, paraffin, 7μ , Masson's stain.



VITAMIN E AND MUSCLE PIGMENT IN THE RAT

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FOUR PLATES (TWENTY-TWO FIGURES)

Rats reared on diets deficient in vitamin E exhibit a brownish pigmentation of the uterus, due to a progressive accumulation of brownish-yellow, insoluble, iron-free granules or globules within the smooth muscle cells (Martin and Moore, '36, '38; Barrie, '38; Hessler, '41; Demole, '41). Similar changes have been observed in smooth muscle cells of the vagina, fallopian tube, seminal vesicle and ureter, and in skeletal muscles generally, but not in other smooth muscle examined (stomach, intestines, urinary bladder, bronchi, spleen, or blood vessels), or in the cardiac musculature (Martin and Moore, '39; Hessler, '41). The testes and abdominal lymph nodes also become pigmented (Martin and Moore, '39). No satisfactory explanation has been offered for these diversified and seemingly unrelated phenomena, which are readily prevented when deficient diets are supplemented with tocopherol.

Reports concerning the reparability of uterine pigmentation are contradictory; it is said to be decreased by tocopherol feeding (Demole, '41), unaffected by tocopherol feeding (Martin and Moore, '39; Hessler, '41), accentuated by resorption pregnancies (Barrie, '38; Hessler, '41) and definitely diminished or even abolished when one or more pregnancies occur coincident with vitamin E therapy (Emerson and Evans, '39; Sweeten, '43). The interesting observation (Hessler, '41) that the pigmented uteri respond normally to drugs which act either upon the autonomic nerve supply or as direct muscle stimulants has been confirmed in this laboratory.¹

More than 5 years ago it became apparent to one of us (K.E.M.) that macrophages, containing pigment globules identical to those seen in intact muscle cells, constituted a prominent feature of this uterine pigmentation. Further study revealed that similar pigment-laden macrophages occurred in skeletal and cardiac muscles, the sex glands, the lymph nodes and elsewhere. Because of the poor delineation of the

¹ We are indebted to Dr. Robert Coons and Dr. Ellen Binkley for carrying out these observations.

pigment in sections stained by hematoxylin and eosin, these cells had apparently been overlooked by other investigators. An extensive study of many organs and tissues of the E deficient rat by a variety of histological techniques has finally permitted certain deductions regarding the nature and distribution of this material.

A preliminary account of these observations and a more complete discussion of the literature on this subject has been presented elsewhere (Mason, '44, p. 135). We have also described, in a previous report (Mason and Emmel, '44), the progressive accumulation of this pigment in macrophages of the ovaries and testes of E deficient rats. This inert material appears to arise in the course of an otherwise normal process of follicular atresia and luteal regression in the ovary, and in relation to degeneration of the seminiferous epithelium. The suggestion that it represents either an abnormal metabolite or a normal metabolite having but a transitory existence in the E sufficient rat receives additional support from the studies reported here, which deal primarily with its mode of occurrence in the smooth, skeletal, and cardiac musculature of the E deficient rat, its subsequent dissemination by means of the lymphatic system, and its retention by these tissues after prolonged periods of tocopherol therapy.

MATERIAL AND METHODS

Histological studies were made upon tissues from forty-two male and 130 female rats reared on an E deficient diet (casein, 20%; cornstarch, 50%; lard, 18%; salts, 2.5%; yeast, 7.5%; cod liver oil, 2%) and from twenty-five control rats of both sexes fed the same diet supplemented with synthetic alpha tocopherol or mixed natural tocopherols,² fed orally. They provided a relatively consecutive series of stages of vitamin E deficiency from the fortieth to the 575th days of life. The majority of females were virgins, others were allowed to resorb one or more litters. Twenty were ovariectomized at different ages, and twenty others were given tocopherol therapy after various periods of deficiency.

Tissues were usually fixed in Zenker-acetic or Zenker-formol. However, Lavdowski's or Susa's fluid used for tissues of rats stained vitally with Tryan blue, Bouin's fluid, neutral formalin, and cold acetone gave good preservation and staining reactions. It was first found that the globular³ form of the pigment stained intensely with either iron hematoxylin or Mallory's basic fuchsin, whereas that sometimes

² We are indebted to Distillation Products, Inc., Rochester, N. Y. for generous supplies of mixed natural tocopherols, and to Merck and Co., Inc., Rahway, N. J. for the synthetic alpha tocopherol used in these studies.

³ Although the term "globule" will be used throughout this report, it should be stated that the pigment in certain situations may have a somewhat granular appearance.

found in diffuse form within cells reacted only with fuchsin. While the pigment also showed affinity for methyl green and other basic stains, its fuchsinophilous staining gave more brilliant results and proved particularly useful in the simultaneous study of pigment and inorganic iron (Turnbull blue or Berlin blue method), and of pigment and Trypan blue, in tissue macrophages. In later phases of this study paraffin sections were stained routinely with Ehrlich's hematoxylin (which does not stain the pigment), counterstained with Mallory's basic fuchsin or Verhoeff's carbol-fuchsin, and destained with acid alcohol. The carbol-fuchsin acid-fast staining, suggested to us by Dr. A. M. Pappenheimer, gave especially brilliant results.

It should be emphasized that other tissue components (keratins, elastins, mucins, erythrocytes, "wear and tear" pigments, mast cell granules) exhibit variable degrees of acid fastness after fuchsin staining. While mast cells can be readily identified on the basis of the uniform size and distribution of their granules, certain of the "wear and tear" pigments are distinguishable from the pigment of vitamin E deficiency largely by virtue of their occurrence in control rats as well as in deficient rats. We find also that these two latter pigments possess the same brownish-yellow fluorescence in paraffin sections (unmounted or mounted with xylol-clarite) examined with the fluorescent microscope.

UTERINE SMOOTH MUSCLE

Pigmentation of the virgin uterus

Particular consideration will be given to sequence of events related to pigmentation of the uterine smooth muscle, which constitutes a prototype for similar changes encountered in smooth muscle elsewhere. The following description is based upon virgin E deficient rats standardized with respect to their initial storage of the vitamin by procedures previously described (Mason and Bryan, '38; Mason, '42).

The uteri of these deficient rats at 40 days of age were normal in all respects. At the sixtieth day small pigment globules appeared in the clear sarcoplasm at each pole of the nucleus of many of the smooth muscle cells. They were more numerous at the eightieth day at which time the uteri, macroscopically, were pale brown in color. Ovariectomy at the twenty-first day of life did not retard the process. Tissue macrophages, demonstrated by vital staining with Trypan blue, were no more numerous in the deficient rats than in the controls at 40, 60, and 80 days of age.

By the 100th day pigment had further increased in muscle cells, pushing the myofibrils more peripherally, and had appeared in a limited

number of macrophages of the muscle layers and the intermuscular connective tissue (fig. 2; compare with fig. 1). These phagocytic cells may have been mistaken by others (Martin and Moore, '39; Hessler, '41) for degenerating smooth muscle cells. Edema, which Hessler ('41) considers an important phase of the picture, was observed occasionally but only in association with proestrous changes in the genital tract.

Continued deficiency merely accentuated the events just described. A progressive accumulation of pigment in muscle cells was paralleled by an increase in the number, size and pigment content of macrophages in the muscularis (figs. 3, 4, 5, 7 and 10). Usually after a year or more of deficiency pigment macrophages also appeared internal to the muscularis and subsequently increased in number throughout the mucosa (fig. 9), eventually intermingling with the iron- and pigment-containing macrophages normally present in the inner zone of the mucosa.⁴ By this time the uteri were dark chocolate brown in color, moderately enlarged, and rather fibrotic when cut; due in part to the increase in macrophages and other connective tissue elements, and in part to fibrotic changes incident to old age. At this stage dystrophy of skeletal muscles was also quite marked, especially in rats originating from litters which recovered spontaneously from paralysis of the late-lactation period (fig. 8, rat A). Even in uteri of virgin rats deficient in vitamin E for 15 months the smooth muscle cells usually appeared remarkably normal except for their bloated appearance due to enormous accumulations of pigment (figs. 5 and 7).

At all stages the histological picture was one of continual production of pigment by otherwise normal muscle cells and transfer of this inert material, in some unknown manner and at a somewhat lesser rate, to macrophages which progressively increased in number and size but were relatively ineffectual in their efforts to autolyze it. Most extreme conditions were encountered after a year or more of deficiency in rats whose postnatal stores of vitamin E were barely sufficient to per-

⁴ A distinction should be made between the macrophages just mentioned, which were invariably devoid of iron, and those containing both iron and pigment globules commonly encountered in the mucosa adjoining the uterine lumen in both deficient and control rats. Since these latter cells appeared first at sexual maturity, remained relatively constant in number in virgin rats, and were suppressed by early ovariectomy but accentuated by pregnancy, they were obviously related to normal cyclic changes in the genital tract. The deeply pigmented spots encountered along the mesometrial border of the uterus, composed of hemosiderin-laden macrophages which throughout the major life span of the rat mark previous sites of implantation, bear no relation to the pigment of E deficiency. They do demonstrate, however, the extent to which macrophages are mobilized in the uterus to take care of cell products and the manner in which the latter, if relatively inert, are retained by macrophages which phagocytize them.

mit recovery from early paralysis (fig. 8, rat A). Macrophages were present in great abundance throughout the muscle layers and often could not be differentiated from muscle cells distended with pigment (fig. 10). In such uteri it was impossible to determine whether or not the muscle cells had undergone any necrosis.

It is recognized that the rat uterus is unusually rich in macrophages (Cappell, '29), which may account for the prominent part which these cells play in the descriptions just given. Undoubtedly some of these escaped into lymphatic channels but the majority, encumbered by pigment, underwent senescence in the uterine connective tissues where their products of disintegration were phagocytized by other macrophages which, in turn, probably suffered a similar fate.

Pigment appeared in the macrophages as discrete globules, somewhat larger and more variable in size than those in the muscle cells but showing the same affinity for either iron hematoxylin or fuchsin; suggesting a complex of pigment and protein. On the other hand, the cytoplasm of the macrophages usually stained diffusely with fuchsin, indicating a possible autolytic liberation of some pigment from this inert complex. Unlike the macrophages of the ovary and testis (Mason and Emmel, '44), those of the myometrium of virgin rats were consistently devoid of sudanophilous lipids and inorganic iron. Trypan blue, injected daily for 6 to 10 days, was phagocytized by many macrophages containing small amounts of pigment or none at all; those containing larger amounts appeared to be refractory to the dye. This inverse relationship between pigment content and capacity to take up Trypan blue was also observed in many other organs and tissues.

Effects of ovariectomy and of pregnancy

It was impossible to compare accurately the pigment picture in uteri of intact rats with that in atrophic uteri of rats ovariectomized at various age periods (1, 21, 28, and 90 to 110 days of life). We can state only that prepubertal ovariectomy did not prevent or significantly retard the process of pigmentation, and that abolition of estrous activity after sexual maturity did not prevent the continued deposition of pigment (figs. 11 and 12). Pigment macrophages did not form a very prominent feature of these atrophic uteri. Uterine macrophages were likewise much less abundant in ovariectomized than in intact control rats. In both ovariectomized and intact deficient rats it seemed that the presence of pigment in smooth muscle cells did not at first invoke any special mobilization of these phagocytic cells but merely brought into greater prominence, through their phagocytosis of pigment, macro-

phages which would otherwise have been residents of the uterine tissues. Subsequently, perhaps secondary to the breakdown of senescent macrophages, there was a gradual increment in their number.

In deficient rats experiencing two or more pregnancy resorptions uterine pigmentation, evaluated grossly and histologically, differed little from that observed in virgin rats of comparable age. However, many macrophages containing hemosiderin appeared in the mucosa and muscularis and in the form of large prominent clusters at the site of implantation as a normal consequence of pregnancy. We could not confirm the observation of Barrie ('38) that pregnancy resorptions accentuate the process of uterine pigmentation.

In rats deprived of E to the sixtieth day of life and then allowed to complete two pregnancies during 100 days of E therapy (10 mg. alpha tocopherol, weekly), the uterine muscle cells and macrophages still contained a small amount of pigment equivalent to that usually encountered at the sixtieth day. In other animals given therapy after longer initial periods of deficiency, rather extensive pigmentation was compatible with, but not significantly modified by, successful gestations; which confirms other observations discussed in the next section. We did not observe the marked reduction in pigmentation reported by Emerson and Evans ('39) and Sweeten ('43).

Uterine pigmentation appears to have no direct relationship to the phenomenon of fetal resorption, since its presence does not interfere with the normal course of pregnancy if sufficient vitamin E is provided. On the other hand, it may be to some degree responsible for the lowered incidence of fertile matings, and increased requirements of vitamin E for successful gestations, which become apparent after the first 6 months of life (Emerson and Evans, '39; Evans and Emerson, '43). We have encountered much difficulty in establishing pregnancies in rats deficient in E for prolonged periods.

Effect of vitamin E therapy

Pigmentation of uterine muscle was prevented by tocopherol feeding if begun prior to the fortieth day of life. On the other hand, comparisons between one uterine horn removed after 7 to 10 months of deficiency and the other horn obtained at autopsy following 5 months of intensive tocopherol therapy (100 mg. mixed natural tocopherols per week), in a group of eight rats, revealed a definite arrest of the process but remarkably little change otherwise (fig. 6). The pigment of muscle cells showed but little reduction over that present at operation. The tendency

of the globules to accumulate in elongated masses at each pole of the nucleus instead of being widely scattered throughout the sarcoplasm made comparisons exceedingly difficult. Grossly, there was no significant reduction of pigmentation. Muscle cells showed neither necrosis nor mitosis. Macrophages of the muscularis and the intermuscular zone were just as abundant as before therapy. Some were senescent, others appeared younger and more active; as if they had recently acquired the products of disintegration of other macrophages. The most satisfactory interpretation of the histological picture was that tocopherol therapy had effected restoration of normal cell functions, resulting in a cessation of pigment production and in an altered permeability of the cell wall such that it no longer permitted escape of the pigment; the latter having in this way been "locked" in the cells sometime after the beginning of therapy.

OTHER SMOOTH MUSCLE

Pigmentation of smooth muscle cells, indistinguishable from that described for the uterine myometrium, was observed in the fallopian tubes, vagina, trabeculae and capsule of the spleen (fig. 20), seminal vesicle, prostate, vas deferens, ureter, bronchi, small intestine, and walls of pulmonary and uterine blood vessels (fig. 13). These changes appeared much later, usually between the sixth and fifteenth months, and are recorded only in the approximate order of their occurrence. The time of appearance and number of pigment macrophages in the splenic pulp (fig. 21) showed an approximate relationship to the extent of pigment production in the trabecular smooth muscle, except in advanced stages of deficiency when it appeared that some of this pigment had an extraneous origin. Frequently the mesothelial cells of the splenic serosa (fig. 21), but never those of other serosal coverings, contained considerable amounts of pigment. Compared with the picture in the uterus, pigment macrophages were relatively sparse in the other smooth muscle sites listed above. This appeared to be related more to the unusual abundance of these phagocytic cells in the uterus in comparison with other organs and tissues. Why smooth muscle in certain locations should be affected before the third month of life, and that in other locations not until the sixth to fifteenth months, has received no adequate explanation. Equally remarkable is the quite different sequence of smooth muscle involvement observed in other laboratory animals, which will be described in subsequent reports from this laboratory.

SKELETAL MUSCLE

Young rats which barely escape the late-lactation paralysis of E deficiency usually show sporadic but transitory lesions of the skeletal muscles (Pappenheimer, '39). In our standardized deficient rats they were observed as late as the fortieth day of life, but had largely disappeared by the sixtieth day. In comparison with the lesions of adult dystrophy described below, there was much more marked proliferation of sarcolemma nuclei, more hyalinization of fibers, and no pigment globules demonstrable in muscle fibers; macrophages were relatively more abundant but the brownish debris which they contained was only weakly fuchsinophilous.

Histological evidence of adult dystrophy appeared about the fourth or fifth month, and outward manifestations (fig. 8) between the seventh and tenth months. However, in rats recovering from severe late-lactation paralysis, histological lesions of the adult type followed those of the earlier type without interruption and a characteristic straddling of the hind legs, poor coordination, and awkward gait were often quite marked by the fifth or sixth month of life. Tocopherol therapy, even when continued for 5 or 6 months, did little more than arrest the process and improve growth and general well being of the animal (fig. 8, rat B). Otherwise the dystrophy progressed slowly such that, by the tenth to twelfth months, the animals had little use of the lower extremities and trunk, and presented the picture described by Ringsted ('35), Einarson and Ringsted ('38), and others (fig. 8, rat A). Such animals, often reared beyond the sixteenth month of life, have provided material for the advanced pictures of E deficiency pigmentation reported here.

Observations were limited chiefly to the diaphragm, abdominal, pectoral and adductor thigh muscles; the latter always showing more marked injury than the other three. They confirmed in general the descriptions of previous investigators (Einarson and Ringsted, '38; Evans, Emerson and Telford, '38; Knowlton, Hines and Brinkhous, '39; Martin and Moore, '39) relative to sporadic proliferation of sarcolemma nuclei, vacuolation of the sarcoplasm, necrosis of muscle fibers with only occasional evidence of hyalinization, relatively little replacement fibrosis, and gross brownish pigmentation. We wish to emphasize two additional phenomena overlooked by previous investigators.

First, the occurrence of pigment globules within the sarcoplasm of many muscle fibers. These globules, identical to those seen in smooth muscle cells, were at first small and arranged in a linear manner between myofibrils. Later they became more irregular in size and ar-

rangement, and surrounded by prominent vacuoles (fig. 14). At this stage muscle striations began to disappear and a slow necrosis of the fiber occurred, usually with little or no evidence of hyalinization.

Second, the widespread occurrence of pigment-laden macrophages compressed between muscle fibers and in small groups at sites of out-spoken necrosis (fig. 14). They appeared to be concerned only in removing products of necrosis of muscle fibers; however, the possibility that some of their pigment was derived from intact fibers by transfer across the sarcolemma sheath could not be ruled out. Their phagocytic capacity for Trypan blue diminished markedly as their content of pigment increased.

The skeletal muscles from rats deprived of vitamin E for periods varying from 5 to 12 months and then given 3 to 5 months of tocopherol therapy showed evidence of arrest of myodegeneration and removal of necrotic remnants of muscle fibers by macrophages. There was relatively little replacement fibrosis or other indication of previous injury, and the histological appearance of the muscle fibers was much improved. Proliferation of sarcolemma nuclei in chain-like rows was frequently noted and ascribed to a phase in the regeneration of muscle fibers. On the other hand, the pigment content of muscle fibers and of macrophages was usually not much less than in corresponding muscles of litter mates sacrificed at the time therapy was begun. This pigment appeared to be a residuum of the previous deficiency period which became trapped in the fibers consequent to the arrest of myodegeneration. Since our observations indicate that pigment was released only in the course of muscle necrosis, it seemed unlikely that alterations in the permeability of the sarcolemma, even though they occurred, played any role in its retention within the muscle fibers. Hence, the factors operating in skeletal muscle fibers during the deficiency state as well as after tocopherol therapy differed in certain respects from those in smooth muscle, even though the ultimate results were quite similar.

CARDIAC MUSCLE

Beginning about the tenth or twelfth month of deficiency pigment globules appeared in cardiac muscle fibers, usually as linear groups located irregularly in the sarcoplasm but sometimes as small clusters at each pole of the nucleus resembling "brown atrophy" in man. During the second year of life more definite histological injury gradually became apparent. Actual necrosis of fibers was less evident, but fibrotic replacement definitely more marked, than in skeletal muscle. Vacuolation of the sarcoplasm was common. Myofibrils remained intact until

necrosis occurred. Some fibers were binucleate, some nuclei were irregularly constricted. Pigment macrophages were invariably present where necrosis of fibers and replacement fibrosis occurred (fig. 15) but were usually absent elsewhere, irrespective of the pigment accumulations in muscle cells. The peripheral portion of the ventricles showed the most marked lesions (fig. 16) but the interventricular septum and atrial musculature were also affected. As far as we are aware, this represents the first evidence that deficiency of vitamin E may invoke lesions in cardiac muscle.⁵ Evidence to be presented at a later date indicates that this is a much earlier and more marked phenomenon in the hamster and cotton rat.

The response to tocopherol therapy closely paralleled that seen in skeletal muscle and, as far as we could ascertain, was subject to the same interpretations. Pigment globules were trapped in the cells in the same manner (fig. 17). The prominent areas of fibrosis so charac-

⁵ There exists an extensive literature ascribing to toxic effects of cod liver oil feeding, heart lesions in rats and other animals closely resembling those described here. In these studies, which are admirably summarized in the later reports of Cox and Roos ('34) and Buraack and Zimmerman ('37), the diets were almost universally lacking in sources of vitamin E and often in other dietary essentials. In a reinvestigation of this problem Buraack and Zimmerman ('37) compared the effects of adding cod liver oil and peanut oil (20% for rats; 27% for mice) to diets with essentially the same composition as that used in our experiments. No source of vitamin E was provided. They describe and picture heart lesions in mice, fed the cod liver oil diet for 150 to 200 days, which are indistinguishable from those encountered in our study. They also describe pigment bearing phagocytes in the myocardium, spleen, liver and skeletal muscles. Mice receiving peanut oil showed none of these changes. The findings in rats, which are known to be less affected by cod liver oil feeding than mice, were similar but less consistent and complicated by acute infections in the animals. Our own observations (not recorded in the present report) indicate that the long period required to induce heart lesions in E deficient rats is not significantly reduced by a high content of cod liver oil (20%) in the diet. The failure of Cox and Roos ('34) to produce any lesions in the heart or any significant changes in the liver, spleen or kidneys of rats reared on similar diets containing even greater amounts of cod liver oil (56%) and likewise unwittingly devoid of vitamin E, is probably due to the fact that the experimental feeding period did not exceed 130 days. There is good reason to believe, therefore, that the myocardial lesions described but not satisfactorily explained by Buraack and Zimmerman (and possibly those reported by earlier investigators) were due actually to an unintentional deficiency of vitamin E in the diets used, and that the absence of such lesions in animals fed peanut oil was due to the vitamin E content of this fat. The heart lesions observed in rabbits fed cod liver oil, but not in those fed equivalent amounts of hydrogenated cottonseed oil (Madsen, '36), are probably subject to the same explanation. Furthermore, there exists some pharmacological evidence unsupported by pathological study (Houchin and Smith, '44) indicating myocardial failure in dystrophic E deficient rabbits.

Due to the unfortunate emphasis placed upon the relation of vitamin E to reproductive processes, and failure to recognize or accept the widespread metabolic dysfunctions and consequent morphological changes induced by its absence and its important functions as an antioxidant, it has frequently not been given due consideration in experimental studies designed to determine the effects produced by the absence of, or an excess of, specific dietary components.

teristic of these lesions showed no alteration after prolonged therapy (fig. 16).

A large series of long term E deficient rats autopsied in 1940 showed gross evidence of cardiac enlargement or dilatation. Certain of these animals were set aside for blood pressure studies which were carried out by Dr. T. R. Harrison and Dr. J. R. Williams at Vanderbilt University School of Medicine by methods developed by them for the study of experimental hypertension (Williams, Harrison and Grollman, '39). During the next 2 years additional groups of rats were studied by the same methods in this laboratory, and a large series of rats were prepared and studied by Drs. Harrison, Williams and Grollman at the Bowman Gray School of Medicine. In none of these studies could there be demonstrated significant alterations in blood pressures attributable to vitamin E deficiency.⁶ The relative heart weights of the rats differed to no significant degree from those of control rats. In this laboratory Dr. Victor Emmel and Dr. Robert Pfaff subsequently carried out a similar series of observations on a group of E deficient and control rats in which hypertension was induced by partial occlusion of the left renal artery. It was found that a state of E deficiency did not in itself cause a significant alteration in blood pressure, nor did it appear to alter the character of the experimentally induced hypertension.

PIGMENT IN OTHER LOCATIONS

Lymph nodes

Examination of superficial inguinal, bronchial, inter-renal, and deep abdominal lymph nodes from rats in progressive stages of E deficiency revealed a variable but continuous accumulation of pigment macrophages, at first within the sinusoids and later in the cellular stroma of the cortex and medullary cords (fig. 18). The nodes eventually

⁶ Our conclusions were based upon averages of twenty to thirty blood pressure determinations carried out during the last 2 to 3 months of life on unanesthetized rats belonging to three experimental groups: (a) E deficient rats 9 to 16 months of age, (b) rats treated in the same manner but given tocopherol therapy during the last 2 to 3 months, and (c) control rats reared on the deficient diet supplemented with tocopherol for equivalent periods. In rats of the first two groups, killed after observations were completed, myocardial lesions were frequently demonstrable. Our inability to demonstrate significant differences in blood pressure in these groups is not in accord with recent observations of Telford et al. ('45) who report a significant lowering of blood pressures in rats deficient in vitamin E for 1 year, which was not further decreased at the second year of life. Their controls, fed a diet of commercial dog chow supplemented with lettuce, showed a comparable though less marked decrease in pressure at 2 years, but not at 1 year, of age. Their animals were anesthetized by barbiturates. It is possible that experimental factors other than the presence or absence of vitamin E may be responsible for these differences in blood pressure.

showed the gross brownish discoloration noted by Martin and Moore ('39), most readily seen in the abdominal nodes near the aortic bifurcation. These macrophages appeared in moderate numbers at the fortieth and sixtieth days of life, coincident with spontaneous remission of early muscle lesions, and increased progressively after about the 100th day. The latter increase seemed to parallel pigment deposition in the ovaries, uteri, skeletal muscles and elsewhere, and presumably represented, in large part, the contents of macrophages which escaped from these sites by way of the lymphatics. Hemosiderin was normal in amount and usually limited to macrophages of the sinusoids, which often contained both hemosiderin and pigment. The pigment appeared first in free macrophages of the sinusoids, then in littoral cells of the sinusoids and in fixed macrophages of the reticulum (fig. 19). The latter cells, which rarely contained hemosiderin and seldom showed much capacity to phagocytize Trypan blue, represented the chief site of pigment accumulation. Engorged with this inert material, and obviously in various stages of senescence, they formed dense clusters or multinucleate masses widely scattered throughout the reticulum. Tocopherol therapy did little more than reduce the pigment content of sinusoidal macrophages and littoral cells, probably reflecting diminished dissemination of pigment from the musculature in general. Pigment macrophages were usually encountered in the thymus also, but never in very large numbers.

Spleen

By the eighth or ninth month of deficiency pigment macrophages appeared in the spleen and gradually increased in number. In contrast to the lymph nodes they appeared first in the splenic pulp and were rarely encountered in the splenic sinuses during the first year of deficiency. As in lymph nodes, the amount and distribution of hemosiderin was essentially the same as in controls of corresponding age. These macrophages appeared coincident with, and during the next 4 or 5 months maintained a proportional relationship to, pigmentation of smooth muscle of the trabeculae and capsule previously described (fig. 20). The mesothelial cells of the serosa, but not that of other organs, frequently contained much pigment probably derived from the muscle cells of the capsule. After more prolonged deficiency the splenic macrophages also appeared to acquire considerable pigment from the general circulation.

Liver, bone marrow and lung

After about a year or more of deficiency small amounts of pigment were demonstrable in Kupffer cells of the liver (fig. 22), in scattered macrophages of the bone marrow, and in macrophages of the lung; the latter were rarely seen in normal lung tissue but were frequent in localized areas of pneumonic involvement. By this time the reticuloendothelial cells of the splenic sinuses and adjacent macrophages were also involved. These findings suggested a general dispersal of pigment by the systemic circulation, either in free form or as the content of phagocytic cells escaping into the blood stream. In some animals, and especially in those given tocopherol therapy after previous deficiency, pigment sometimes appeared as concentric rings around fat globules within liver cells.

Kidney

After deficiency of 10 months or more globules of pigment appeared in the epithelium of the proximal convoluted tubules. At later stages they were also encountered in the loops of Henle, in the collecting tubules, and in macrophages of the connective tissue. More information is necessary to establish whether or not this material is actually excreted through the kidney. Only in a few animals did we observe the parenchymatous degeneration described by Martin and Moore ('39).

DISCUSSION

These studies, together with the earlier observations of Martin and Moore ('39) and others, clearly point toward a widespread metabolic dysfunction of smooth, skeletal, and cardiac muscle in the vitamin E deficient rat, characterized by the production of an unusually inert combination of pigment and protein. While muscle tissue represents the major site of its production, this pigment occurs at many other sites in the E deficient rat and is not always bound to protein. When it appears in association with extensive fatty degeneration of cells as in the ovary (Mason and Emmel, '44), and in adipose tissue after feeding diets high in cod liver oil (Dam, '44; Dam and Mason, '45) it seems to be chemically or physically bound to lipids and is indistinguishable from the so-called "ceroid" occurring in relation to nutritional cirrhosis in rats (Lillie, et al., '42; Endicott and Lillie, '44; Popper, Gyorgy and Goldblatt, '44). Its occurrence in certain epithelial tissues is under investigation in this laboratory and will be reported upon at a latter date.

This pigment, in its staining reactions and fluorescence, closely resembles the "wear and tear" pigments encountered in the adrenal cortex and elsewhere in the presumably normal organism, but differs from them in its sites of formation, general distribution, and prevention by dietary additions of vitamin E. It gives no dopa reaction for melanin, differs from porphyrins in its fluorescence, is devoid of inorganic iron and appears to have neither an exogenous nor a hematogenous origin. Its remarkable inertness, resistance to autolysis by macrophages, and capacity for prolonged retention in muscle cells without serious effect upon their physiological responses, suggest an abnormal intermediary product of cell metabolism or a metabolite having but a transitory existence in the E sufficient organism. It has been obtained from tissues as a brownish-yellow amorphous powder resembling, in its solubilities and fluorescence, products formed by the acid hydrolysis of proteins (Moore and Wang, '43). Chemical identification of this pigment should provide valuable leads to the metabolic role of vitamin E.

There is also need for a better understanding of the sites and modes of origin of this pigment in tissues exclusive of the musculature of the E deficient organism, and the manner in which its occurrence may be influenced by dietary fats. Fitzhugh et al. ('44) have referred briefly to pigment in skeletal and smooth muscle cells and in adjacent macrophages in rats fed diets containing rancid lard. These changes, which did not occur if corn oil replaced the lard, were recognized as similar to those of E deficiency but emphasis was placed upon the lack of rancidity in corn oil as compared to cod liver oil, rather than upon the possible protective action of vitamin E in the corn oil. Recent evidence (Dam, '44; Dam and Mason, '45) indicates that, unless adequate vitamin E is provided, a similar pigment occurs in developing fat cells obliged to phagocytize unsaturated fatty acids presented to them as a result of high cod liver oil feeding. On the other hand, there is no reason to believe that the small amount of cod liver oil (2%) present in our diet bears and particular relationship to the lesions described. This is in keeping with the observations of others that muscular dystrophy can be produced in rats and in rabbits (Mackenzie, Mackenzie and McCollum, '39, '41) by E deficient diets from which fat has been rigidly excluded.

It remains to be determined whether this pigment ever occurs in the musculature, and at other sites described in this report, under other experimental conditions where an adequacy of vitamin E is assured. In view of the similarity between this pigment and ceroid, it may be stated that the diets used for producing nutritional liver cirrhosis in rats have quite universally been deficient in vitamin E, and that the

ceroid arising in this condition is also quite widely disseminated throughout the reticuloendothelial system. Hepatic cirrhosis without ceroid has recently been reported and attributed to the nature of the fat in the diet (Endicott, Daft and Sebrell, '44). It seems possible, however, that inclusion of alpha tocopherol in the diets used in the latter study may provide a more satisfactory explanation of the results obtained.

It seems rather unlikely that muscle pigmentation and macrophage responses as pronounced as those described for the E deficient rat, especially those involving the uterine myometrium, occur under other experimental conditions. For this reason, and on the basis of similar findings in other laboratory animals deprived of vitamin E (unpublished studies), accumulation of acid-fast pigment in the musculature and elsewhere appears to be a reliable and useful criterion of vitamin E deficiency with possibly greater specificity and practical significance than the biochemical criteria (increased O_2 consumption, alterations in concentration of creatine, electrolytes and water) which, unfortunately, have been established only for dystrophic skeletal muscle. This morphological criterion has one disadvantage; namely, it does not permit sharp differentiation between an existing deficiency state and a state of deficiency followed by therapy. On the other hand, this recording of previous deficiency in the tissues of the organism might, under certain conditions, be utilized to advantage.

SUMMARY AND CONCLUSIONS

1. These studies confirm and extend observations of previous investigators (Martin and Moore, and others) concerning gross discoloration and intracellular accumulations of an inert, yellowish-brown, iron-free pigment in uterine and other smooth muscle and in skeletal muscles of the vitamin E deficient rat.

2. By virtue of its acid-fast and other staining characteristics, and its fluorescence, this pigment was followed histologically in a wide variety of tissues of rats deficient in vitamin E from the fortieth to the 575th days of life. It arose primarily but not exclusively in the smooth, skeletal, and cardiac musculature, and was subsequently acquired by tissue macrophages which showed very little ability to degrade it but, to a limited degree, effected its dissemination through the lymphatic system.

3. In the smooth muscle cells of the uterus, where most striking changes occurred, pigment globules accumulated gradually from the sixtieth day onwards until the cells became markedly distended with globules. No cell necrosis could be demonstrated. Pigment was con-

stantly released to macrophages which, lacking the ability to effectively autolyze it, progressively increased in number and size and, for the most part, became stranded in the muscularis and mucosa. Neither ovariectomy nor pregnancy resorptions significantly modified this uterine pigmentation. After prolonged deficiency similar, but usually less extensive, changes were seen in the smooth musculature of the trabeculae and capsule of the spleen, fallopian tubes, vagina, seminal vesicle and prostate, vas deferens, small intestine, bronchi, ureter and certain blood vessels.

4. In dystrophic skeletal muscle, pigment globules usually surrounded by vacuoles were invariably present in fibers showing other evidence of histological injury. In contrast to the situation in smooth muscle, pigment was relatively less abundant and appeared to be released to macrophages only during, or subsequent to, actual necrosis of the muscle fibers.

5. Myocardial lesions, not previously observed in E deficient rats, were frequently observed after a year or more of deficiency. They resembled those observed in skeletal muscle except for their later appearance and the marked fibrotic reaction in areas of necrosis. They were not associated with any significant alterations in blood pressure.

6. Tocopherol therapy, extending over periods of 3 to 5 months, appeared to arrest pigment production and cell necrosis, but caused very little reduction in pigment content of the muscle fibers (whether smooth, skeletal or cardiac) or in the number and pigment content of associated macrophages. The pigment appeared to have been trapped within the muscle fibers by certain changes incident to restoration of their metabolic functions. The macrophages, for the most part, became stranded in the adjacent connective tissue where senescence, disintegration and re-phagocytosis occurred.

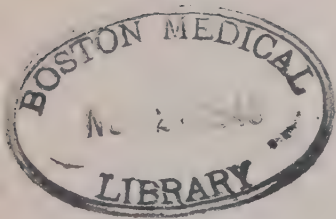
7. Lymph nodes everywhere, to a variable degree, showed a progressive accumulation of pigment within the free macrophages of the sinusoids and the fixed macrophages of the cellular stroma. After prolonged deficiency pigment was encountered in macrophages of the splenic pulp, thymus, and bone marrow, in Kupffer cells of the liver and in the proximal convoluted tubules of the kidney.

8. The pigment appears to represent an abnormal metabolite arising secondary to disturbed cell functions. Its value as a histological criterion of vitamin E deficiency is discussed.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Portion of circular muscle layer from transverse section of uterus of control rat 150 days old, fed an E deficient diet supplemented with synthetic alpha tocopherol, showing normal appearance of the smooth muscle cells. Iron hematoxylin and basic fuchsin. $\times 270$.

2 Corresponding region of uterus from vitamin E deficient rat 100 days old, showing early appearance of small pigment globules staining intensely with haematoxylin within the sarcoplasm of many muscle cells. Several deeply stained macrophages (M) can be seen at the left. Iron hematoxylin and basic fuchsin. $\times 270$.

3 Similar area from deficient rat 170 days old, showing increased accumulation of pigment in muscle cells and in macrophages (M) of the intermuscular connective tissue. The latter have increased in size and stain intensely with fuchsin. Here, as in all preparations of the myometrium of virgin E deficient rats, inorganic iron could not be demonstrated by the hydrochloric acid-potassium ferricyanide test. Turnbull blue and basic fuchsin. $\times 270$.

4 Smilar area from deficient rat 280 days old, showing abundance of pigment globules in muscle cells between which may be seen numerous dark staining macrophages somewhat smaller than those present in the intermuscular connective tissue at upper left. Iron hematoxylin and basic fuchsin. $\times 270$.

5 Similar area from deficient rat 458 days old, showing more extensive accumulations of pigment in muscle cells. Dark staining structures at left are clusters of macrophages in the intermuscular connective tissue. Iron hematoxylin and basic fuchsin. $\times 270$.

6 Similar area from right uterus of rat deficient in vitamin E to the 235th day of age and then given tocopherol therapy (50 mg. mixed tocopherols, weekly) for 150 days. The left uterus removed before therapy began showed a stage of pigmentation intermediate between that seen in figures 3 and 4. The smooth muscle cells still contain an abundance of pigment which tends to be more condensed in elongated masses at each pole of the nucleus and gives the muscle cells a more slender appearance than before therapy. Pigment macrophages, mostly at lower left and upper right, are as abundant as before therapy. Ehrlich's hematoxylin and carbol fuchsin. $\times 270$.

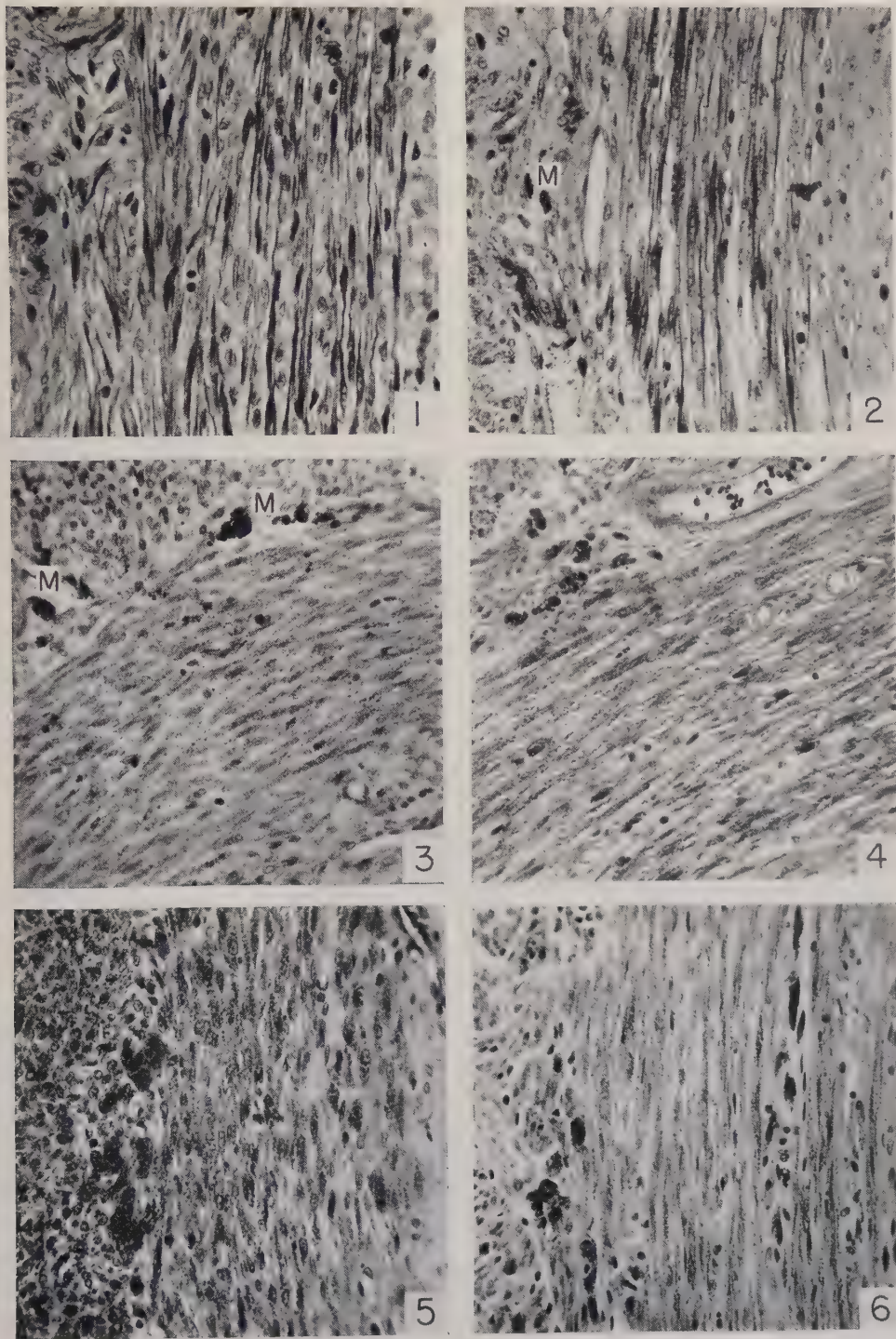


PLATE 2

EXPLANATION OF FIGURES

7 Enlarged view from different portion of the uterus shown in figure 5. Cells of circular muscle layer (right) are greatly distended with pigment globules which are smaller and more uniform in size than those within the large macrophages at left. Ehrlich's hematoxylin and basic fuchsin. $\times 606$.

8 Photograph of rats showing two stages of muscular dystrophy.

Rat A spontaneously recovered from late lactation paralysis and was reared on the E deficient diet to the 360th day of age. Note the paralysis and spasticity of the hind extremities. The flank and trunk musculature was greatly atrophied but the animal was able to drag itself about the cage without great difficulty.

Rat B had a similar history except that mixed natural tocopherols were fed daily from the 245th to 360th days. Dystrophy was arrested at the straddle stage present at the beginning of therapy; there was also improvement in muscle strength, body growth and general appearance. Histologically, the uteri resembled the condition shown in figure 6.

9 Low power view of uterus of rat A, figure 8, showing extensive pigmentation in longitudinal musculature and intermuscular connective tissue (1), circular muscle layer (2), and periphery of the mucosa (3). The uterine lumen (4) which stains faintly, is outlined in ink. The deeply stained structures at top center (5) represent areas of fibrosis around blood vessels of the mucosa. Otherwise, deeply staining material represents pigment in muscle cells and macrophages. Iron hematoxylin and basic fuchsin. $\times 38$.

10 Portion of same uterus at higher magnification. Intermuscular connective tissue (left) contains prominent clusters of pigment macrophages. In the circular muscle layer at right it is difficult to differentiate between elongated macrophages and muscle cells distended with pigment globules. In advanced stages such as this it is difficult to say whether or not actual necrosis of smooth muscle cells has occurred. Iron hematoxylin and basic fuchsin. $\times 270$.

11 Uterus of deficient rat ovariectomized at 110 days of age, after two resorption pregnancies, and continued on the diet to the 272nd day. The atrophic uterus presents a picture similar to that shown in figure 9. Only the phagocytic cells in the mucosa adjacent to the uterine lumen gave a positive reaction for inorganic iron. Turnbull blue and basic fuchsin. $\times 38$.

12 Enlarged view of circular layer of smooth muscle in figure 11. Pigment is abundant in muscle cells and in macrophages within and adjacent to the muscle layer. Macrophages are much less abundant than in intact rats at similar stages of deficiency (compare with figure 4). Iron hematoxylin and basic fuchsin. $\times 270$.

13 Portion of mesometrial zone from uterus of deficient rat 458 days old. Two sections through a branch of the uterine vein are connected by an oblique section through the superficial part of the vessel wall (left center). The smooth muscle cells of the blood vessel wall contain large numbers of pigment globules. There are also numerous pigment macrophages in the surrounding connective tissue. A portion of the muscularis of this same uterus is shown in figure 5. Ehrlich's hematoxylin and carbol fuchsin. $\times 410$.

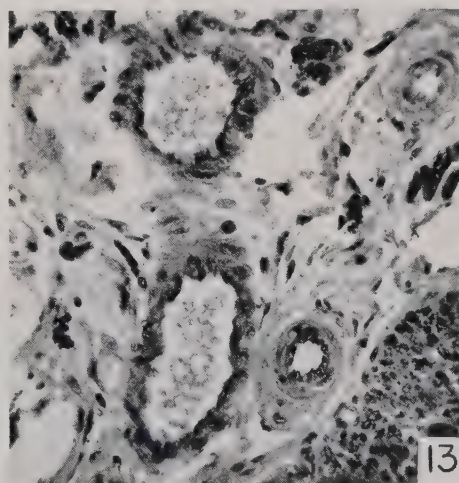
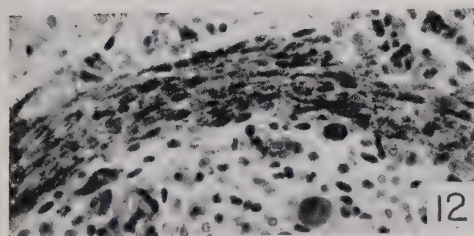
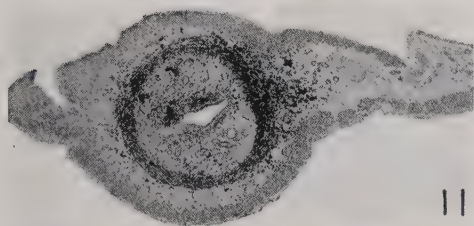
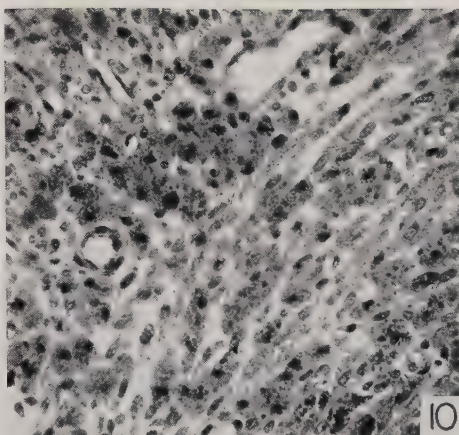
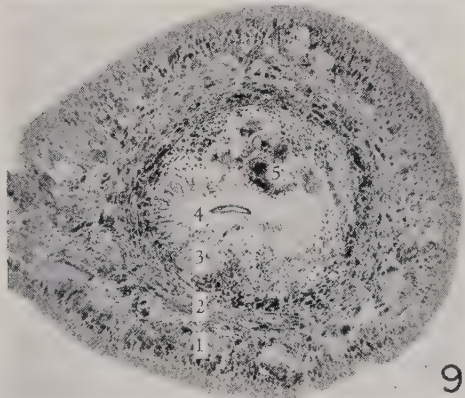
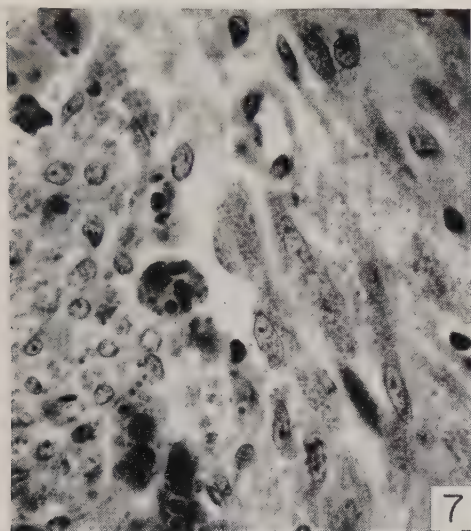


PLATE 3

EXPLANATION OF FIGURES

14 Hyaline necrosis in adductor thigh muscle of deficient rat 348 days old. Fibers in upper half and at lower margin of figure contain large numbers of pigment globules (usually surrounded by vacuoles), chain-like formations or clusters of sarcolemma nuclei, and varying degrees of necrosis without hyalinization. Beneath a twisted hyalinized fiber (which were encountered only occasionally) are several large elongated macrophages and other smaller ones (right center). Several fibers in lower half of figure are relatively normal. Ehrlich's hematoxylin and basic fuchsin. $\times 430$.

15 Portion of left ventricle of deficient rat 524 days old, showing large number of pigment macrophages in connective tissue replacing necrotic muscle fibers. Though not demonstrated in the photomicrograph, most of the muscle fibers contained many small pigment granules (like those shown in figure 17 at higher magnification) and striations were usually quite evident. Ehrlich's hematoxylin and basic fuchsin. $\times 430$.

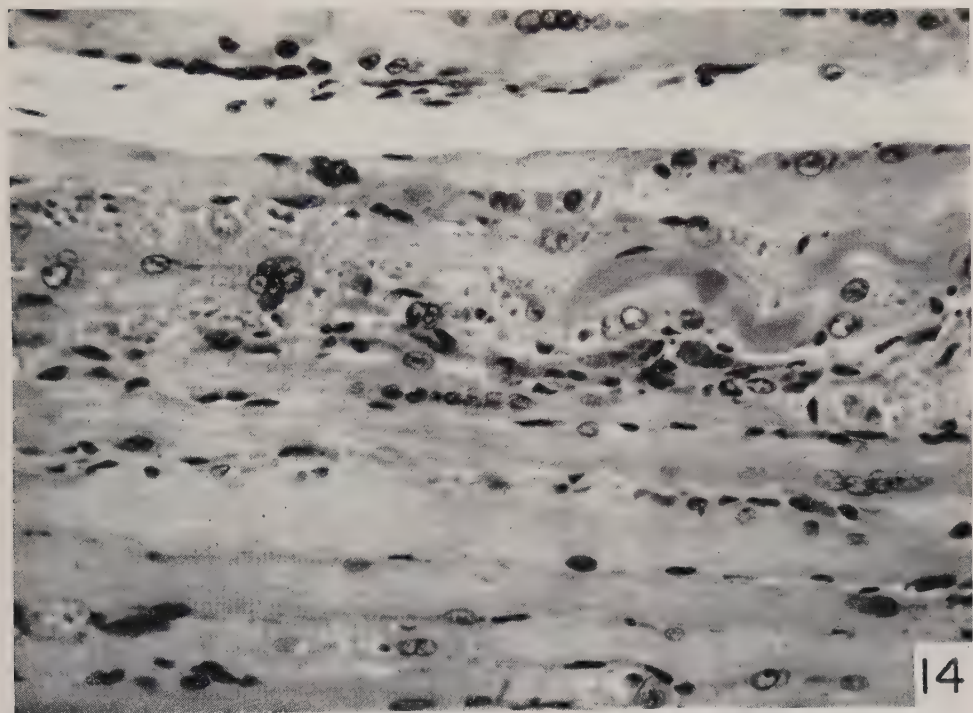


PLATE 4

EXPLANATION OF FIGURES

16 Peripheral portion of left ventricle from rat deficient in vitamin E to 285 days followed by 150 days of tocopherol therapy (100 mg. mixed natural tocopherols, weekly) showing areas of replacement fibrosis near surface of myocardium (right) containing large numbers of deeply stained macrophages barely visible at this magnification. Ehrlich's hematoxylin and basic fuchsin. $\times 38$.

17 Portion of myocardium of figure 16 at higher magnification to show distribution of pigment globules in cardiac muscle cells. Note cluster of three pigment macrophages. $\times 270$.

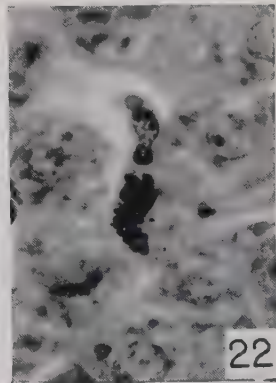
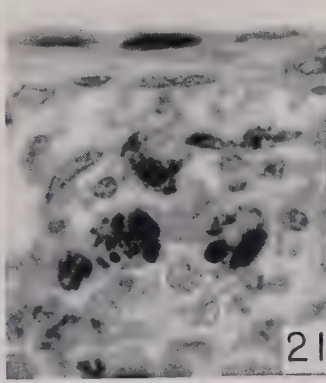
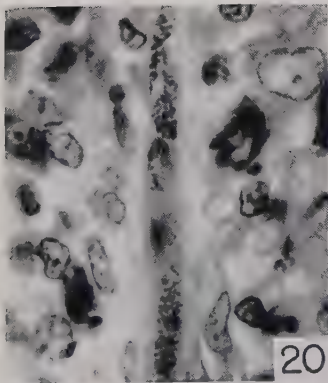
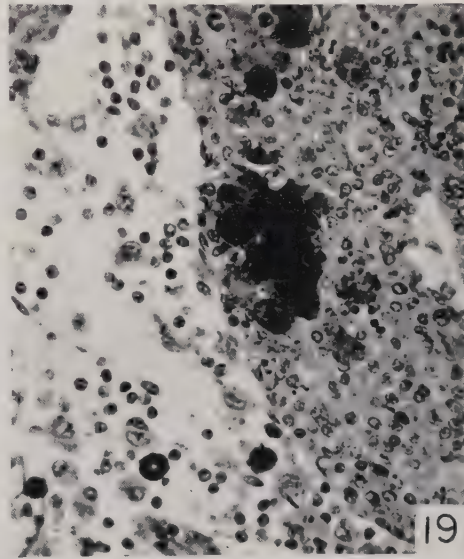
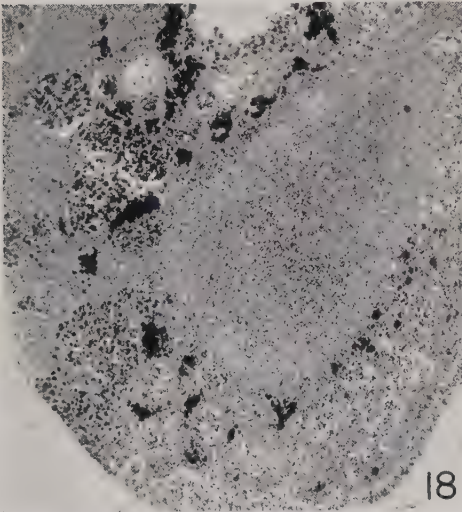
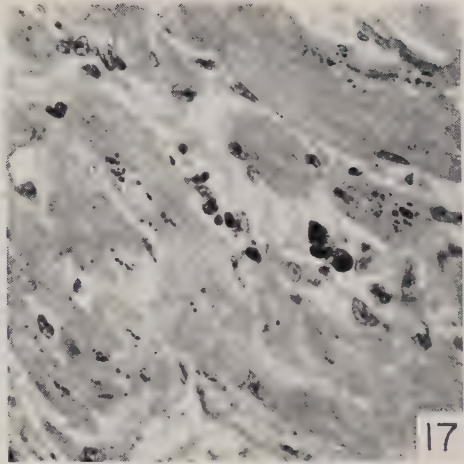
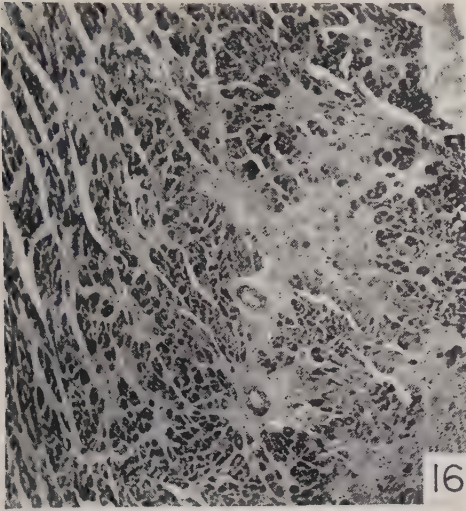
18 Abdominal lymph node of deficient rat 360 days old, showing many clusters of pigment macrophages in the reticular stroma, and large numbers of free macrophages in the sinusoids. Small amounts of inorganic iron as well as pigment globules were demonstrable in macrophages at upper left; otherwise, the deep staining material represents pigment. Turnbull blue and basic fuchsin. $\times 38$.

19 Margin of sinusoid from another section of lymph node shown in figure 18, showing cluster of twelve large fixed macrophages and six individual macrophages in the adjacent stroma. Many smaller free macrophages with pigment globules, difficult to identify in the photograph, are dispersed throughout the sinusoid at left. The three dark cells in row at bottom are mast cells. Ehrlich's hematoxylin and basic fuchsin. $\times 270$.

20 Portion of spleen of deficient rat 360 days old showing two smooth muscle cells of trabeculae (center) loaded with pigment globules, and four pigment macrophages in splenic pulp. Ehrlich's hematoxylin and basic fuchsin. $\times 940$.

21 Periphery of same spleen, showing pigment in two mesothelial cells and in four macrophages of the splenic pulp. Ehrlich's hematoxylin and basic fuchsin. $\times 940$.

22 Pigment globules in two Kupffer cells from liver of same rat. Ehrlich's hematoxylin and basic fuchsin. $\times 940$.



THE NORMAL MYELOGRAM IN ALBINO RATS

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THREE PLATES

In the course of investigating experimental blood dyscrasias in albino rats, it became necessary to determine the normal myelogram of the strains of rats which we used. Thirty-three healthy male and female rats of the Wistar, Osborne and Mendel, and New Haven strains weaned at 3 weeks and fed stock diet were studied for this purpose. Rats were 3 weeks to 2 years of age.

The work of Kindred ('42) indicates that in rats, femoral marrow is quite representative. Our own observation of sections of vertebral, femoral, and tibial marrow from several thousand rats examined in this laboratory in other investigations have led us to the same conclusion. For these reasons, femoral bone marrow was used for marrow smears in this study. Tissue sections of marrow were prepared from the femur and from dorsal vertebra. The relative proportion of active marrow to marrow space was determined by a modification of Kindred's ('42) method. Leucocyte counts, differential counts, and hematocrit determinations were carried out on blood taken from the tail for the purpose of excluding rats with blood dyscrasias. Gross and microscopic post mortem examinations were made to exclude rats with other diseases.

EXPERIMENTAL METHODS

Blood studies were made by standard methods using freely flowing tail blood. Total leucocyte counts were carried out in duplicate. Differential counts were made on Giemsa-stained smears by the meander method. Two hundred to 500 cells were identified. Hematocrit was determined with the van Allen hematocrit tube using 1.3% sodium oxalate.

After the blood studies were done, the rats were killed by decapitation or by exposure to illuminating gas. The right femur was stripped of soft tissue and was opened in mid-shaft. Thin smears were prepared by diluting aspirated femoral marrow with plasma (Endicott, '45) and

were stained with Giemsa stain. Five hundred to 1,000 cells were classified. The left femur and a portion of dorsal spinal column were fixed in Orth's fluid, decalcified in formic acid, embedded in low viscosity nitrocellulose, sectioned at 8μ and stained by a modified Romanowsky technique (Endicott, '45).

Gross autopsy was performed and heart, lungs, liver, pancreas, spleen, adrenals, kidneys, and a portion of thigh muscle were fixed in Orth's fluid, embedded in paraffin, sectioned, stained with van Gieson's stain and with hemalum azure-eosinate, and examined. Animals showing gross or microscopic pathology were discarded.

The sections of bone marrow were studied quantitatively to determine the relative volume of the marrow space occupied by hemopoietic cells. This was done by outlining the various components of the marrow (marrow cells, fat, vasa, bone trabeculae) on thin paper with a projection apparatus. The projection apparatus consisted of a microscope, lamp and special table of convenient working height (see drawing). The microscope was arranged under the table and the image was projected through the glass insert in the table top directly onto the thin paper upon which the drawings were made. It was found helpful to construct a light-tight housing extending from the microscope ocular to the glass top to screen out stray light. Four drawings were made from each rat — one from vertebral marrow and three from femur (mid-shaft, distal shaft, and epiphysis). Each drawing represented a strip of marrow 200μ in width, 8μ thick, and the inside diameter of the bone in length. The area in the drawings occupied by each of the components of marrow was determined by counting the spaces which they filled when viewed by light transmitted through finely ruled graph paper. The area occupied by marrow cells in the drawing was assumed to be proportional to the actual volume in the living marrow. The values (active cellular marrow percent) given in the chart are the average of the areas occupied by marrow cells divided by the total areas of marrow space in the drawings.

OBSERVATIONS

Preliminary studies of marrow smears convinced the authors that the usual detailed classifications of marrow cells were neither desirable nor accurate with our methods. This conclusion was based on several observations. Firstly, the cells of the granulocytic series in the rat mature by the formation at an early stage of "ring" or "doughnut" nuclei in which there is a gradual enlargement of the central cytoplasm-filled space and condensation of nuclear chromatin into progressively

coarser particles. The cytoplasm matures at an independent rate by gradual loss of basophilia and acquisition of specific granulation. Since the rates of nuclear and cytoplasmic maturation of any given cell are often not parallel, and since the maturation is gradual, any attempt at arranging the granulocytic series into definite promyelocyte, myelocyte, and metamyelocyte stages is highly artificial and subject to extreme variation from differences in interpretation. Secondly, some of the marrow cells are dislodged from the stroma with more difficulty than others so that they appear rather irregularly in smears and never in proportion to their occurrence in marrow as seen in sections. This is particularly true of the megakaryocytes, mast cells, stroma cells, and vascular endothelial cells.

For these reasons we made the following rather simple classification of the marrow cells:

I. Neutrophilic granulocytes.

- (a) Older forms (metamyelocytes and segmenters). These cells are characterized by pale cytoplasm with neutrophilic granules and ring form or segmented nuclei with coarsely divided chromatin.
- (b) Younger forms (promyelocytes and myelocytes). These relatively large cells are characterized by blue cytoplasm with azurophilic or neutrophilic granules and with relatively large nucleus with more or less finely divided chromatin, with or without nucleoli, and with or without the "ring" form.

II. Eosinophilic granulocytes. All forms are included in the single group.

III. Red cell series. All recognizable nucleated precursors of erythrocytes are included in this group.

IV. Lymphocytes. Large, medium and small lymphocytes are included in this group.

V. Blasts. All the large cells with basophilic granule-free cytoplasm, large nuclei with finely divided chromatin and with nucleoli are included in this group.

VI. Other cells. In this category are included the megakaryocytes, mast cells, plasma cells, reticuloendothelial cells, basophils (very rare), stroma cells, vascular endothelial cells, and unidentified cells.

In table 1 are given the values obtained in eleven rats under 60 days of age and in twenty-two rats older than 60 days. The groups are separated on the basis of our finding a consistent and marked difference in

the marrow and to a lesser extent in the blood of these two groups. No significant difference was noted with respect to the sex or strain in this small group.

TABLE 1
Hemograms and myelograms.

	RATS LESS THAN 60 DAYS OLD (11)		RATS MORE THAN 60 DAYS OLD (22)	
	Mean and standard deviation ¹	Index ²	Mean and standard deviation ¹	Index ²
<i>Peripheral blood</i>				
Hematocrit	45.1 ± 5.82		45.6 ± 3.90	
Total leucocytes/cu. mm.	6,120 ± 1,650		14,570 ± 5,110	
Neutrophils/cu. mm.	1,580 ± 1,310		3,180 ± 1,968	
Eosinophils/cu. mm.	30 ± 40		240 ± 171	
Lymphocytes/cu. mm.	4,520 ± 1,450		10,840 ± 4,332	
Nuc. red cells/cu. mm.	95 ± 105		(1 rat showed 40, all others 0)	
<i>Bone marrow smears</i>				
Neutrophilic				
Metamyelocytes and segmenters %	16.2 ± 8.36	14.1	34.5 ± 6.19	28.5
Myelocytes and promyelocytes %	7.7 ± 6.3	6.7	9.2 ± 2.31	7.6
Eosinophilic leucocytes (all types) %	4.8 ± 1.9	4.2	8.3 ± 5.30	6.9
Total granulocytes %	28.6 ± 14.5	24.8	52.0 ± 9.46	43.0
Nuc. red cell series (all types) %	53.5 ± 11.5	46.4	30.8 ± 2.52	25.5
Myeloid/erythroid ratio	0.62 ± 0.504		1.93 ± 0.93	
Blasts %	1.3 ± 0.85	1.13	1.9 ± 0.57	1.57
Lymphocytes %	14.8 ± 7.68	12.9	13.3 ± 4.27	11.0
Other cells %	1.7 ± 1.00	1.47	2.0 ± 1.08	1.65
<i>Bone marrow section</i>				
Active cellular marrow %	86.8 ± 2.16		82.7 ± 9.12	

¹ Standard deviation was calculated from the equation, $S.D. = \sqrt{\frac{S(x^2)}{n-1}}$.

² The index was calculated by the equation, $\text{Index} = \text{Mean \% in smear} \times \text{active cellular marrow}$.

DISCUSSION

In the young albino rat, there occurred a marked alteration in the myelogram between the age of 3 weeks and 9 weeks. The marrow of the weanling rat was predominantly erythropoietic while that of rats over 2 months of age was predominantly granulocytopoietic. The change appeared to occur largely between the age of 1 month and 2 months. In some rats, however, it had already been accomplished at

the age of 1 month. This change is presumed to occur in response to a change in the demands of the organism.

Related changes occurred in the peripheral blood. The leucocyte count rose and the polychromatophilic erythrocytes and nucleated red cells which are regularly present in very young rats disappeared.

The degree of cellularity of the marrow decreased somewhat with advancing age. In weanling rats, the marrow spaces of the femur and vertebra were almost completely filled with marrow cells (86.8%) while in 2-year-old rats, the marrow cells occupied only 79.0% of the marrow space. In the tables we include an "index" of total quantity for each of the various cellular components of the marrow. This index is the product of the mean percent value in the marrow smear and the mean percent of active cellular marrow in sections as determined by the drawing method. We propose to use this "index" as a base-line for semi-quantitative studies of the bone marrow in blood dyscrasias. It offers a practical means of correcting the differential count in hypocellular or hypercellular marrows so that intelligent comparisons can be made. It must be emphasized, however, that it is an index and not a true measurement. It does not take into account the variation in cell size and makes no measurement of actual rate of cell division or rate of discharge of cells into the blood. Unfortunately, no practical method for making these measurements is available at present.

A survey of the literature reveals no studies of rats of similar age groups. Stasney and Higgins ('35) studied twenty-four Wistar rats 6-8 months of age. Their classification differs somewhat from ours in that the granulocyte series is divided into myeloblast, leukoblast, promyelocyte, myelocyte, metamyelocyte and granulocyte. In their myeloid-erythroid ratio they include myeloblasts while we do not include these cells since we attempt no separation of the blast cells into myeloblast, lymphoblast, monoblast, etc. Calculating from their tables for femoral marrow with myeloblasts excluded we find their mean myeloid/erythroid ratio to be 1.79/1 as compared to our 1.69/1 for rats over 2 months old. Their values for the erythroid series is somewhat higher than ours and their values for lymphocytes is lower. These discrepancies may be due to differences in the strains of rats or may be due to the difference in technique. Stasney and Higgins used touch preparations.

SUMMARY

The hemogram and myelogram of albino rats of two age groups are presented. In rats under 1 month of age, the marrow was found to be predominantly erythropoietic while in rats over 2 months of age the

marrow was found to be predominantly granulocytopoietic. A modification of Kindred's method of estimating the relative volume of active marrow is presented along with the values obtained by its application. A simple apparatus for sketching from projected microscopic slides is described.

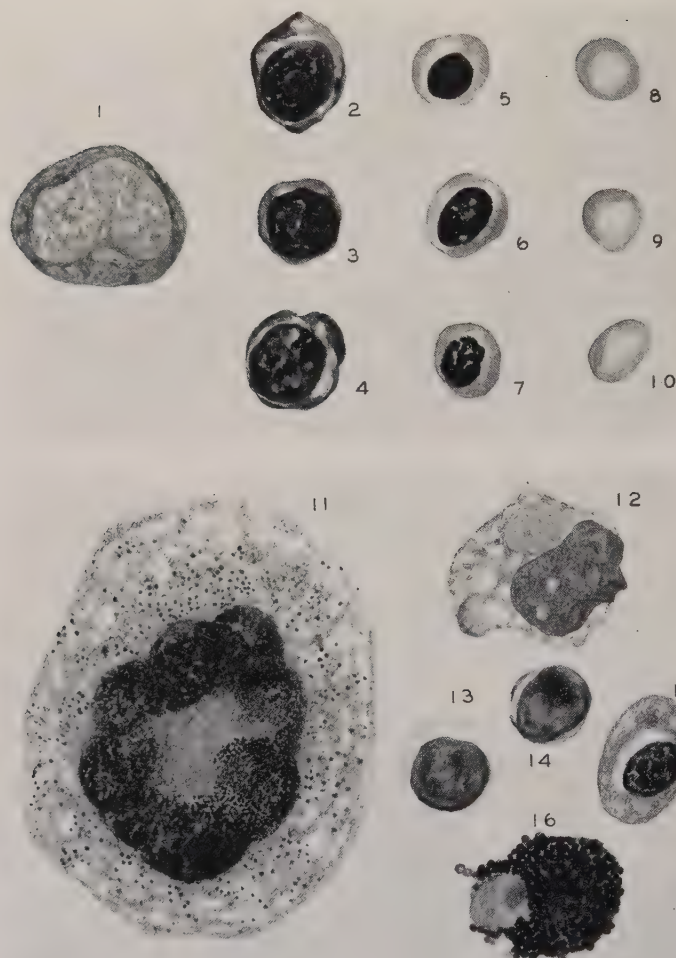
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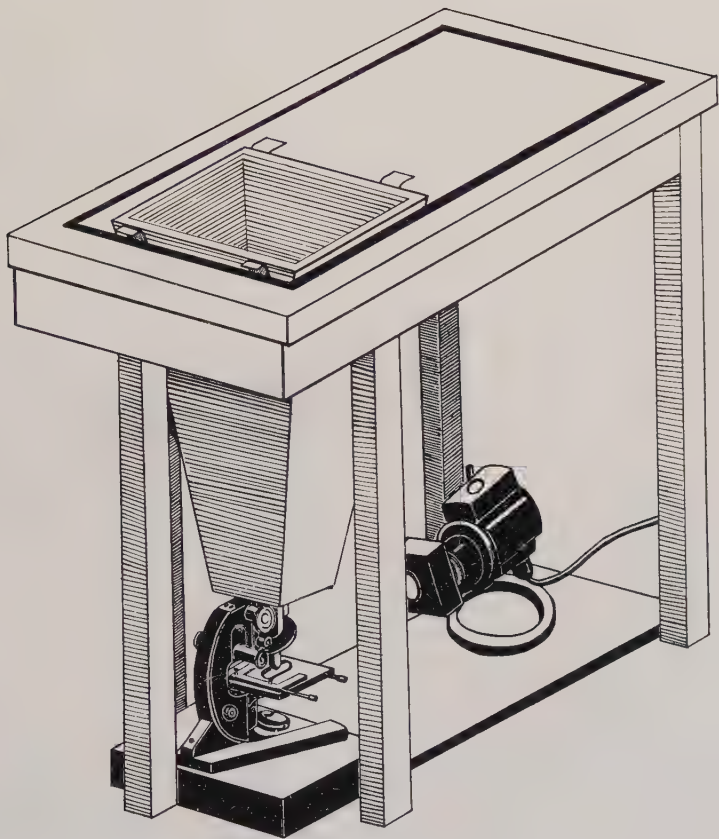
INEZ DEMONET

Drawings of cells in marrow smear, Giemsa stain, $\times 1000$. (1) Blast cell; (2), (3) neutrophilic premyelocytes; (4) neutrophilic myelocyte; (5), (6) neutrophilic metamyelocytes; (7), (8), (9) neutrophilic segmenters; (10), (11) eosinophilic leucocytes.



INEZ DEMONET

Drawings of cells in marrow smears, Giemsa stain, $\times 1000$. (1)-(7) incl. nucleated precursors of erythrocytes; (8)-(10) incl. erythrocytes; (11) megakaryocyte; (12) reticulo-endothelial cell containing erythrocyte; (13), (14) lymphocytes; (15) plasma cell; (16) mast cell.



Drawing of projection apparatus.

REPORT OF TWO UNUSUAL VENOUS ABNORMALITIES (LEFT POSTRENAL INFERIOR VENA CAVA; POST-AORTIC LEFT INNOMINATE VEIN)

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FOUR FIGURES

In the course of the routine dissections in this Anatomy Laboratory, two cases with unusual venous abnormalities were noted.

1. LEFT POSTRENAL INFERIOR VENA CAVA

This was a white male subject who died of Cardiovascular Renal disease at the age of 79. The cadaver was in a fair state of preservation when examined. No unusual findings were recorded throughout the dissection with the exception of a left postrenal inferior vena cava.

The right common iliac vein joined with the left on the body of the fifth lumbar vertebra, behind the left common iliac artery (fig. 1). The left inferior vena cava thus formed, passed upwards, partly on the bodies of the lumbar vertebrae, partly on the left psoas major muscle, lying to the left of the aorta as far as the body of the second lumbar vertebra where it received the renal veins. Here the vena cava passed abruptly, across to the right, passing in front of the aorta in the position usually occupied by the left renal vein. On reaching the right side of the aorta, the vein bent sharply and passed upwards to the right of the aorta in the normal position of the inferior vena cava.

The vein presented, as described, a normal pre-renal segment, in addition to a left-sided post-renal segment, and utilized an inter-renal segment to traverse the midline. Since the right renal vein entered the transverse limb on the right, and the left renal vein entered correspondingly on the left, the renal veins on both sides were short and equal in length.

The left testicular vein, and the left adrenal vein both entered the vena cava directly. The former opened into the post-renal segment about half an inch below the renal vein, the latter into the beginning of the inter-renal segment. On the right, both these veins entered the right renal vein.

A right post-renal inferior vena cava opening into the right extremity of the inter-renal segment was present as a very small channel to the right of the aorta. This vein was traced downward as far as the side of the body of the fourth lumbar vertebra where it disappeared by passing backward between the right psoas and the bone. This channel also received the fourth right lumbar vein.

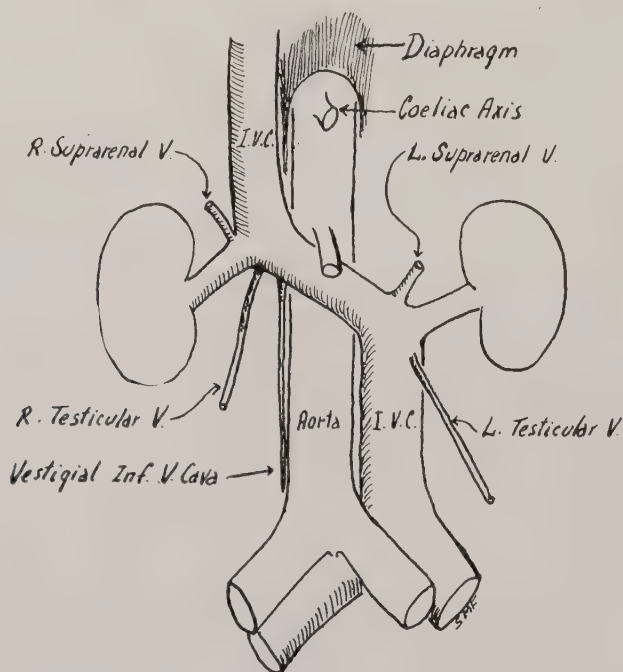


Fig. 1 Sketch of specimen exhibiting a left postrenal vena cava. (A photograph of the cadaver is available).

Discussion

A case almost identical in type, and certainly identical in morphogenetic features was reported in 1937, by Blasingame and Burge ('37) who also reviewed the literature on the subject. The present case differs from theirs only in minor detail, since their subject presented two left renal veins, and a much shorter vestigial right post-renal inferior vena cava, as well as deviation of the aorta to the right. The anatomical explanation of this anomaly would seem to lie in the persistence of the post-renal segment of the left supracardinal vein. This maintained its anastomotic connections cranially to the renal subcardinal anastomosis, and caudally to the iliac anastomosis of the postcardinal veins.

From the age of the subject we infer that this anomaly is compatible with longevity.

2. POSTAORTIC LEFT INNOMINATE VEIN

This was a white male subject who died of nephritis at the age of 66. The cadaver was well preserved. No unusual findings were recorded throughout the dissection with the exception of a postaortic left innominate vein and the concomitant venous derangements.

In the neck the external jugular veins on both sides were absent. The anterior jugular veins were large, especially that of the left side, which joined the right by a prominent interjugular connection through Burns' space (fig. 2). The blood from both anterior jugular veins was delivered chiefly to the right subclavian vein, but, on the left, a smaller

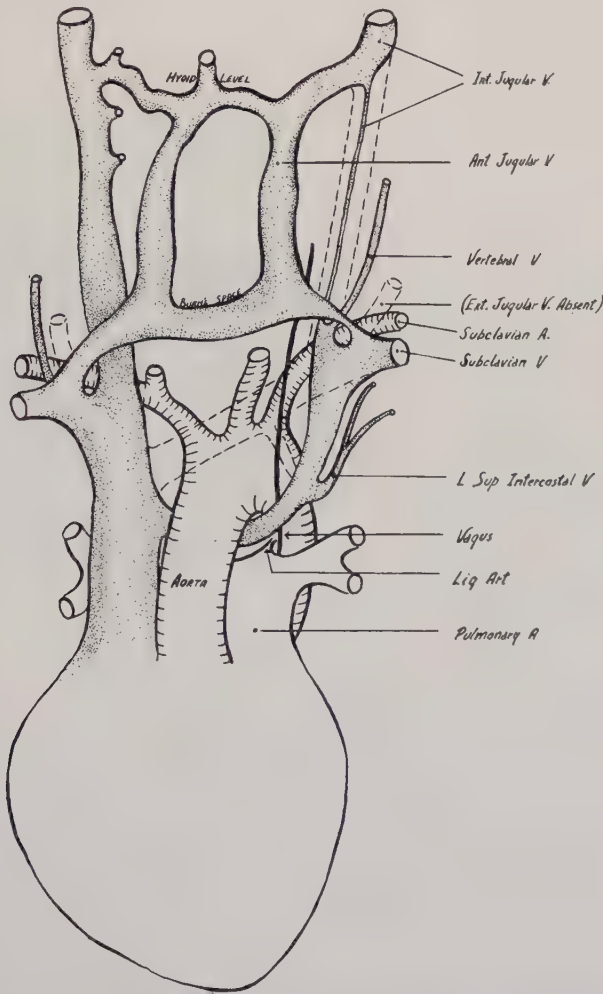


Fig. 2 Sketch of second specimen to show the relations of the anomalous post-aortic left innominate vein, and the major features of venous drainage from the head. Dotted lines indicate positions of absent normal structures.

corresponding channel was present which allowed some drainage of the anterior jugular to the left subclavian vein.

The internal jugular vein on the right was normal in size and position, as well as in its union with the right subclavian vein to form the right innominate vein. On the left side, the internal jugular vein emptied into the anterior jugular at the level of the hyoid bone. Below this, its course was represented only by a fine channel lying on the left side of the common carotid artery. Traced downward this small channel joined with the left vertebral vein to form a short common stem, which united almost at once with the left subclavian vein to form the left innominate vein. The caliber of the left subclavian and left innominate veins was about equal, so that the two appeared directly continuous.

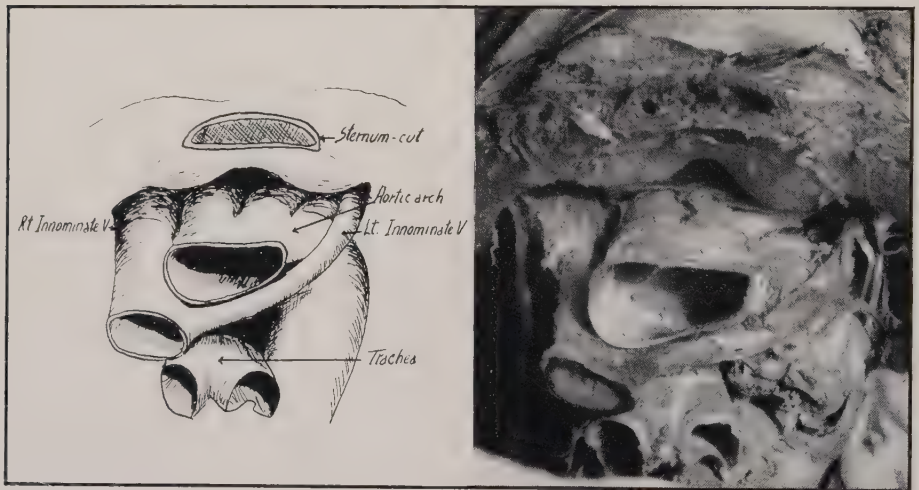


Fig. 3 Detailed sketch and photograph showing relations of left innominate vein.

The left innominate vein passed downward into the thorax, lying at first in front and to the left of the left subclavian artery (fig. 3). It then crossed the transverse part of the aortic arch, and slipped under the concavity of the arch to join the right innominate vein. In this part of its course the vein passed superficial to the left vagus nerve, medial to the ductus arteriosus and above the left pulmonary artery, entirely extrapericardial in position.

No other related or unrelated anomalies were noted in this subject, and certainly nothing which could account for this unusual arrangement.

Discussion

A similar case was reported by Martin ('31). In his specimen, however, there were other intracardiac abnormalities. Furthermore, his description was based on an autopsy specimen removed from the cadaver so that he could not be certain of the course and connections of the left innominate vein.

A case essentially similar to the present one was reported by Walter ('31). I am aware of no other records of similar cases.

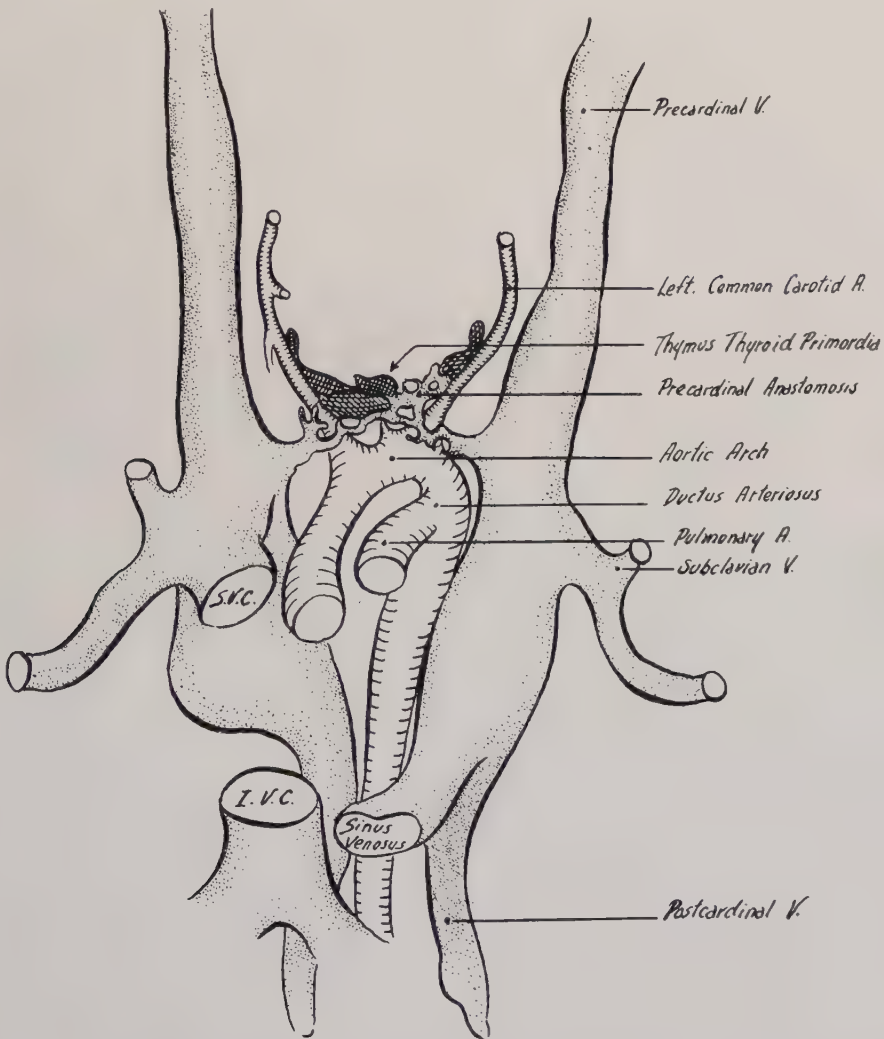


Fig. 4 Sketch of Human embryo no. 492 in collection of Carnegie Institute of Embryology. C. R. length 16.8 mm. Coronal view of injected specimen.

The morphogenetic explanation of this anomaly is not clear. While it might be related to anomalous branches of the linguo-facial vein as described by Lewis ('02, '09) it would seem hazardous to speculate too far particularly since the abnormal vein was wholly outside the pericardial wall.

In a personal communication, Dr. George W. Corner ('45) states that the "drawing of embryo no. 492, showing the capillary net which is the precursor of the left innominate vein, makes it clear that at such a stage a few stray sprouts which happened to wander behind the aortic arch could easily provide a precursor of your anomalous vessel." Embryo no. 492 is shown in figure 4, a copy of an original illustration by Dorcas Hager Padget.

Possibly the anomalies in the jugular veins of the left side were secondary in an attempt to deflect the main mass of head and neck blood to the right, since the anomalous left innominate vein was subject to compression with each heart beat.

CONCLUSION

1. A case of a post-renal left inferior vena cava is presented in a 79-year-old white male.
2. A case of a post-aortic left innominate vein is reported in a 66-year-old white male.

ACKNOWLEDGMENTS

I am deeply indebted to Dr. George W. Corner of the Carnegie Institute of Embryology for his helpful suggestions; to Dorcas Hager Padget, the medical illustrator, who has long been associated with Dr. Walter E. Dandy, for her admirable drawings from which my figures 3 and 4 are taken, and to Miss H. Z. Anderson of this Department.

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ORBITAL DEFORMITY AND VISUAL IMPAIRMENT DUE TO LOPPING OF THE COMB IN THE CHICKEN

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TWO FIGURES

The anatomical features which function to protect the eye globe in the chicken include the bony orbit, the lacrimal bone, the orbital membrane (Chamberlain, '43), the nictitating membrane, the two lids and the sclerotic plates (Nelson, '42). In normal birds these structures afford adequate protection to the eye. It has been noted in Single-Comb White Leghorn males at this Laboratory that excessive comb growth together with lopping of the comb may produce asymmetry of the cranium, deformity of the orbit, dislocation of the eye globe and impairment or complete obstruction of vision on the affected side. The bird may thus be hindered, if not incapacitated, in functions for which either binocular or bilateral vision is favored.

Buckner, Insko and Martin ('32) attributed excessive comb growth in cockerels, when confined, to a lack of direct sunlight. The actual cause of lopping has not been reported. Mueller and Hutt ('42) found no evidence of heredity upon the direction of the lop in pullets.

The amount of deformity caused by the comb impinging upon the supraorbital margin of the orbit is apparently closely related to the weight of the comb. In some cases which showed marked deformity of the bony orbit and dislocation of the globe, the excised combs weighed as much as 100 gm.

The base of the comb, on the lopped side, bears much of the weight of the comb and impinges upon the supraorbital rim, orbital membrane and on the globe (fig. 1). Due to the downward and inward displacement of the orbital rim and the consequent insufficiency of the accessory orbital membrane, the orbit is rendered too small to accommodate the eye globe (fig. 2). Thus, the inadequate protective adnexa allow the globe to protude from the dorsal side of the orbit. In some cases the displacement of the globe position is sufficient to place the pupil below the edge of the lower eyelid, thus completely obscuring vision of that eye. In less marked cases, a deviation of the optical axis occurs. Technically this syndrome would be termed a mechanical strabismus.



Fig. 1 Lopping of large comb with impingement on the supraorbital rim. The displacement of the dorsal side of the globe outwardly places the pupil below the edge of the lower lid.

Fig. 2 Deformed skull, ventral view. Note the right supraorbital rim and the medial septum.

A secondary feature which accompanies this deformity is an increase in the acuteness of the corneal curvature manifested by an excessive bulging. The change of the curvature from the normal may be entirely mechanical, produced by a distortion of globe shape. The weight of the comb would tend to compress or flatten the globe on the horizontal axis. Somewhat contrary to this explanation is the possibility that an actual increase in intraocular pressure, due to the weight of the comb, does take place. The secondary glaucoma may conceivably result also from an interference with normal circulation, particularly of drainage of the anterior chamber due to the dislocation of the globe. It is perhaps significant that the atrophy syndrome which follows true glaucoma in the human eye has not been noted to occur from this cause in the chicken.

The syndrome described has not been observed in females.

SUMMARY

The lopping of combs of excessive size causes a deformity of the bony orbit with displacement of the eye globe. The result is that the optical axis is deviated from the normal to the extent that vision is impaired or completely occluded.

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TECHNIQUE FOR PARATHYROIDECTOMY IN PIGEONS

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FOUR FIGURES

As early as 1904 Vincent and Jolly noted that removal of the parathyroids in mammals is complicated by the fact that much or most of this tissue is inconveniently imbedded in the thyroids. This operation therefore involves a certain amount of thyroid trauma, and completeness of the operation usually remains in question until the animal is killed and the thyroid region sectioned. Observations on the comparative anatomy of the parathyroids of birds, including one species of dove, were made by Forsyth ('08). Nearly 20 years later Nonidez and Goodale ('27) recorded the removal of the parathyroids of fowl. These investigators found that four parathyroids are located close to, but outside of, the thyroid; and that in the fowl some accessory parathyroid tissue is usually found imbedded in the thymus. Removal of the four visible parathyroids did not lead to tetanic convulsions in fowl because accessory tissue maintained parathyroid function. Campbell and Turner ('42) found no trace of parathyroid tissue in serially sectioned thyroids of four 7-day-old chicks. Benoit and associates ('42) parathyroidectomized ducks but the birds apparently survived one day only.

In pigeons the two pairs of parathyroid glands are located outside of the capsule of the thyroid and entirely separate from it and from the thymus. Parathyroid tissue attached to the posterior end of the left thyroid was found in only two of 150 of our operated birds. A dozen thyroid glands from young and adult Carneau pigeons were found to contain no parathyroid tissue (Riddle, Rauch and Smith, '45). Although the parathyroids of pigeons are not readily accessible, the fact that they are completely removable without injury to the thyroid makes this animal almost unique for certain studies on parathyroid function. The technique for parathyroidectomy in pigeons was developed in order to determine the relation of the parathyroids to cyclic changes in medullary bone and to estrogenic action on the plasma calcium (Riddle, Rauch and Smith, '44; '45). It seems that an illustrated description of this technique is warranted.

MATERIALS

Parathyroidectomy was performed on several breeds of pigeons before it was learned that the White Carneau is especially suitable because of its large size and the shape of its furculum. More working space and better visibility were thus obtained.

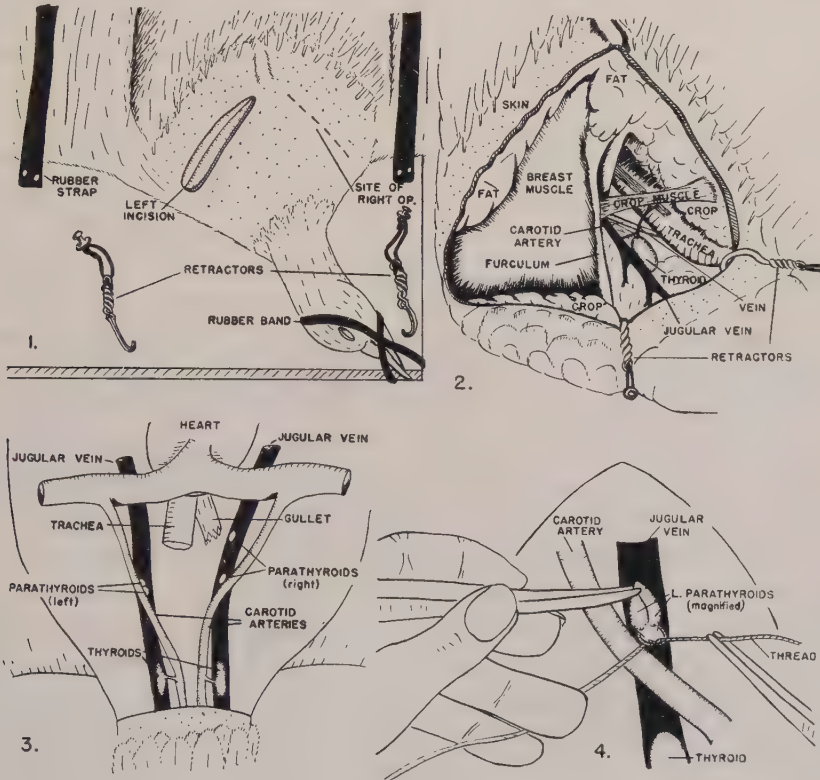
A $30 \times 35 \times 2$ -cm. board, with rubber straps to hold the wings and legs, is used. The head end of the board, supported about 3 cm. higher than the opposite end, is directed toward the operator. A Spencer "Universal" type lamp is so placed that a beam of light is readily directed into the thoracic aperture as the operation proceeds. Spectacle-type binocular magnifiers (A. H. Thomas no. 6353) are advisable since the glands are quite small (1-3 mm.) and so situated that good visibility may mean the difference between success and failure in the completeness of the operation. Other required instruments include two wire retractors (fig. 1), one curved dissecting scissors, one tenotomy scissors, two straight-pointed dissecting forceps (13 cm.), two mosquito forceps (hemostatic) with straight points, no. 50 cotton thread, Michel's suture clips and clip forceps, and sizes 0-3 cotton pellets (Richmond Dental Mfg. Co.).

OPERATIVE TECHNIQUE

It was found best to remove the glands in two operations, left and right, separated by an interval of 1 week. In this way operative trauma is less likely to be severe and the tetany following the loss of the second pair of glands may be more easily controlled. As a precaution 0.1 ml. dihydrotachysterol (A. T. 10, provided by the Winthrop Chemical Company) has been injected into each bird from 2-24 hours before the second operation. Nembutal (25 mg. per kg. body wt.) is very satisfactory as an anesthetic and is slowly injected into the radial vein. Sterile technique is not necessary since pigeons are not as susceptible to infection as mammals; however, the instruments should be kept in 5% cresol solution or other disinfectant during the operation.

Removal of the glands. After plucking the feathers from the area, an incision about 6 cm. long is made through the skin on the left side starting from the shoulder and stopping just short of the sternum (fig. 1). Using large cotton pellets moistened with physiological saline and held in the two forceps, the crop is gently separated from the furculum and pulled toward the midline. A muscle extends from the furculum to the crop (fig. 2); it is sometimes necessary to retract this muscle but it should not be cut. The shiny translucent interclavicular air sac is also exposed and must be punctured and torn open. After the air sac

is opened, the bird's head is fastened securely by means of a rubber band; this band is placed around the back of the head, crossed over the lower beak, and placed around the end of the operating board (fig. 1). There is little danger of strangulation because the bird can now breathe through the opened air sac. Two retractors are placed so that one holds the trachea toward the midline and the other, with a cotton pellet under it, holds the crop and skin out of the line of vision (fig. 2). During the



Figures 1 to 4

operation the exposed breast muscle should be covered with a piece of cotton moistened with saline.

A small vein runs from the trachea to the posterior end of the thyroid (fig. 2). With the mosquito forceps clamping both its ends this vein can be gently severed; about 1 minute later the forceps can be released without subsequent bleeding. Fat and soft connective tissue are cleared from the region posterior to the thyroid with the dissecting forceps; this exposes the carotid artery and the jugular vein. Figure 3 shows

diagrammatically the location of the parathyroids, thyroids, carotid arteries and jugular veins.

On the left side the aureate ellipsoidal parathyroids are usually found together within the same capsule; they lie on the jugular and just under the carotid artery at the point where these vessels cross. With small cotton pellets, held in one or both forceps, the gland is partly freed from the jugular; when the distal end of the glands can be held in one forceps this procedure is facilitated, but care must be taken not to tear the jugular. When the glands can be lifted, as in figure 4, a noose of no. 50 cotton thread is slipped over them and tied, dissecting forceps being used to handle the thread; the mosquito forceps are then used to tighten the knot. Each ligature should have two knots. As the glands are lifted out the tenotomy scissors are used to cut between the glands and the knot, and also to cut the loose ends of thread close to the knot. The retractors are then removed, the entire area moistened with saline, the bird's head unstrapped, the edges of the skin brought together with Michel's suture clips, and the closed incision swabbed with 95% alcohol. The bird's mouth is swabbed out with cotton to remove accumulated mucus, and the bird placed in a constant temperature chamber at 30°C., to prevent shock.

It was stated above that the bird is given an injection of 0.1 ml. A. T. 10 before the second operation. The procedure for the removal of the right parathyroids is similar to that just described except that the gullet is retracted instead of the trachea and the bird's head is strapped down on the left side of the board. The position of the parathyroids on the right side varies considerably. Occasionally the right parathyroids are within the same capsule but more often (when they are separated by no more than 3 mm.) they are joined by a strand of connective tissue; in other cases, as in figure 3, they are entirely disconnected and rather widely separated.

Post-operative care. The birds are kept overnight in a constant temperature cage with access to water. When they regain consciousness, usually within a few hours, they are given orally either 0.2 gm. calcium in the form of gluconate in water or a capsule containing 1 gm. aluminum hydroxide (gel) to prevent the tetanic convulsions that otherwise prove fatal. The use of aluminum hydroxide for this purpose was suggested by Dr. Ephraim Shorr; this has proved to be the most satisfactory post-operative treatment for these birds. Most of the birds can be kept in excellent condition by feeding one capsule containing 1 gm. of the aluminum hydroxide per day. Birds may also be maintained with cal-

cium gluconate (2 or more doses daily) and occasional supplementary injections of 0.1 ml. A. T. 10 when convulsions seem imminent.

Related observations. Two to three operations can be performed in an hour. Some idea of the completeness of the operation can be obtained by examination of the extirpated glands under binoculars at the time of the operation. After some practice with this technique it is possible to obtain completely parathyroidectomized birds in nearly all cases. Only one-eighth of all of our operated birds showed any residual parathyroid tissue when examined with binocular magnifiers at autopsy. Any suspicious tissue was sectioned for histological study. A check on the completeness of the operation was obtained by measurement of total plasma calcium at regular intervals (Riddle, Rauch and Smith, '45). The numerous instances of tetany (with occasional deaths) in birds not given adequate postoperative care further indicate the completeness of these operations.

Parathyroid size in confined pigeons fed standard diets will probably be found to vary greatly depending upon the amount of sunlight to which the birds have been exposed (Hollander and Riddle, '45). For example, six young pigeons reared under slight-light exposure to sunlight had parathyroids with an average weight (for the four glands) of 28.5 mg.; six similar sets of glands from young birds which had been exposed to direct sunlight averaged 3.4 mg. Glands from five adults, also exposed to direct sunlight, averaged 3.7 mg.

SUMMARY

The parathyroid glands of pigeons are entirely separate from the thyroids and the thymus. A technique for the complete removal of the parathyroids of pigeons by a two-stage operation is described. Operated birds can be kept alive for long periods by feeding aluminum hydroxide or calcium gluconate, the latter supplemented by A. T. 10.

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A RARE TYPE OF ANOMALOUS OPHTHALMIC ARTERY IN A NEGRO

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ONE FIGURE

According to the standard anatomical textbooks, the ophthalmic artery normally arises from the internal carotid immediately below the anterior clinoid process, passes through the optic foramen, and provides the main blood supply to the contents of the orbit. It is well known, however, that the orbit and its contents not infrequently can be supplied with blood by branches from the anterior division of the middle meningeal artery rather than by the internal carotid via its ophthalmic division. Yet in such instances one commonly finds some of the intraorbital branches (usually the central artery of the retina) arising, as normally, from the internal carotid artery. Instances where the blood supply of the orbital contents is derived wholly from a branch of the middle meningeal artery apparently are exceedingly rare. The present communication deals with such an example.

On the left side of the head of an adult male Negro we found the ophthalmic artery arising from the middle meningeal and not at all from the internal carotid artery (fig. 1). The course of the middle meningeal artery along the floor of the middle cranial fossa was normal. The anomalous ophthalmic artery arose from its anterior branch not far from the foramen spinosum. Curving medialward, it entered the orbit through the superior orbital fissure, passing along the course of the not infrequent anastomosis between the middle meningeal and lacrimal arteries. Within the orbital cavity the vessel assumed the path and distribution of a normal ophthalmic artery, excepting only the central artery of the retina. The latter arose from the very beginning of the lacrimal artery and passed deep to the optic nerve to enter its sheath on the infero-medial aspect.

On the right side of the head there was found no evidence of any vessel arising from the internal carotid save its terminal branches, but as the middle meningeal artery was destroyed during dissection we were unable to confirm a duplication of the anomaly. There is reason

to believe, however, that the condition was essentially similar to that found on the left side.

In a search of the literature, the writers have found only three instances of the occurrence of this particular anomaly. Dubrueil (1847) recorded two of these cases. In these the ophthalmic artery arose from the anterior branch of the middle meningeal artery and passed into the

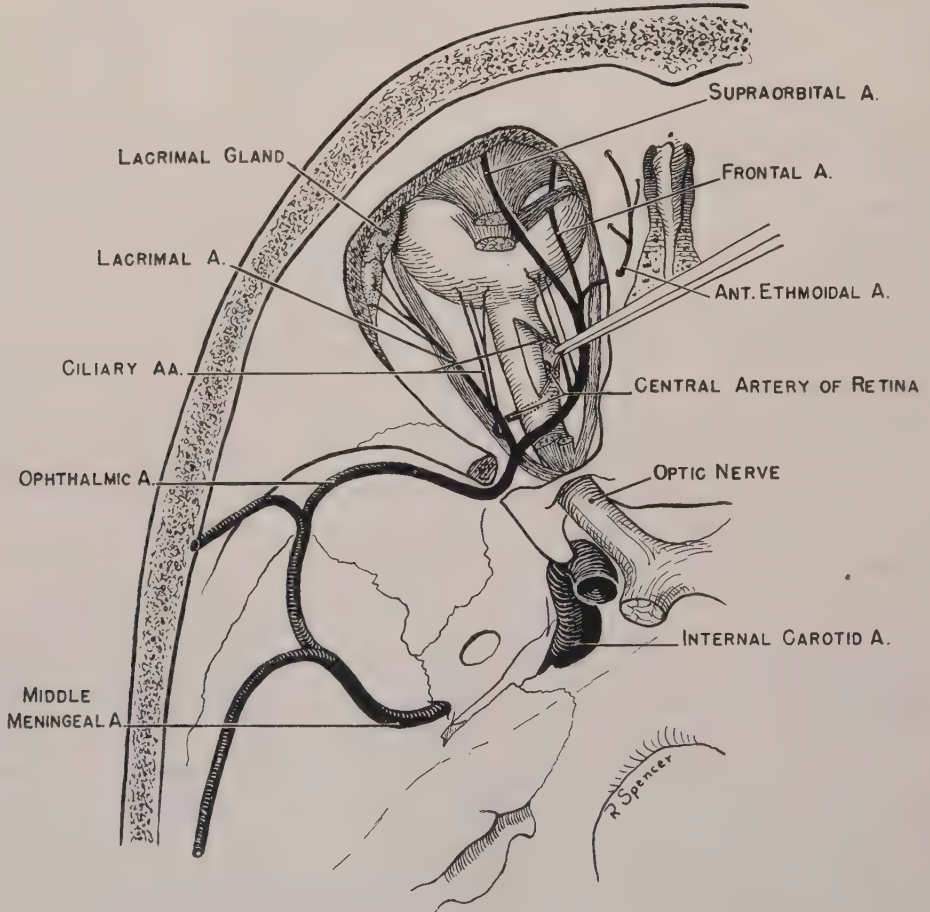


Fig. 1 Origin of the entire ophthalmic artery from the middle meningeal artery in an adult male Negro.

orbit via the supraorbital fissure. Its distribution in the orbit was normal. Adachi ('28) in a study of 142 heads, found one case in which the ophthalmic artery arose from the middle meningeal. He makes no reference to the origin of the central artery of the retina. This case, therefore, must necessarily remain as a doubtful example of the anomaly under discussion.

There are other investigators (as Blandin, Chanmugam, Curnow, Krause, Tiedemann) who have described variations of this anomalous ophthalmic artery; but in every instance some portion of the blood supply to the orbit, notably the central artery of the retina, arose from the internal carotid artery.

Meyer (1887) described and reviewed anomalies of the blood vessels supplying the orbit. In this work he used Krause's axiom that anomalies result from abnormal development of normal embryonic anastomoses. This led him to believe that if the root of the ophthalmic artery degenerated it would be possible that all the blood could come from the middle meningeal artery through persistence of an embryonic anastomosis. Padget, in work as yet unpublished, finds that in the human embryo the ocular branches (ciliary and central artery of the retina) arise, after a complicated series of secondary anastomoses, from the internal carotid and that the orbital branches of the adult ophthalmic artery arise from the primitive stapedia (a large component of which forms the middle meningeal artery). Her study further reveals that an anastomosis between these primitive orbital and ocular branches later takes place. This then explains the not infrequent finding of large branches of the ophthalmic artery arising from the middle meningeal artery. Normally the primitive arterial connection between the middle meningeal and the definitive ophthalmic artery is lost. In the case herein described one would assume that there was disappearance of the most proximal portion of the ocular branch (stem of the definitive ophthalmic artery) of the internal carotid with persistence and further development of the primitive arterial connection between the middle meningeal artery and the definitive ophthalmic artery.

We are greatly indebted to Dr. W. L. Straus, Jr. for aid and criticism given us in the preparation of this paper, to Mrs. D. H. Padget for help and for permission to refer to her unpublished study of the development of the cranial arteries, and to Miss Rowena Spencer for the accompanying illustration.

SUMMARY

A case is reported wherein the ophthalmic artery arises wholly from the anterior branch of the middle meningeal artery. This anomaly appears to be exceedingly rare. A possible embryological explanation is offered.

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A NOTE ON THE PISO-TRIQUETRAL JOINT

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The usual description of the piso-triquetral joint, as given in most of the popular textbooks of anatomy in English, states that this space is completely separated from the cavity of the radio-carpal joint. However, two of the anatomical atlases consulted illustrate a very definite communication between the piso-triquetral and radio-carpal joints:—thus Toldt, vol. 1, figs. 446 and 448; and Grant, vol. 1, fig. 62. Spalteholtz, vol. 1, p. 209, states “The joint slit of the articulatio ossis pisiformis is in about a third of the cases connected with the articulatio radio carpea.” Similarly, Morris, p. 322, states “Its articular cavity is usually separate from the general cavity of the wrist.” The textbook statements were found to conflict with the findings in a great majority of unselected dissecting room cases.

The piso-triquetral joint is enclosed by a small, but usually loose and tough capsule attached near the periphery of the articular surfaces of the pisiform and triquetral bones. The fibers of this capsule fuse above with one fascicle of the ulnar collateral ligament and laterally with the medial extension of the transverse and volar carpal ligaments. The other attachments of the pisiform bone need not be considered in connection with this note. The joint may be opened by incising the capsule along its lower and medial borders, and displacing the pisiform bone upwards and laterally. Twenty-eight subjects (both hands) were routinely examined in this way. In forty-three of the fifty-six hands examined it was found that the capsule was incomplete along the upper and lateral margins permitting a probe to pass easily into the radio-carpal joint. In all but two of these forty-three instances this communication took the form of a round or slightly flattened aperture 1 mm. to 3 mm. in diameter, and in the other two the communication consisted of a membrane perforated by two or three small apertures each of which admitted a bristle that entered the radio-carpal joint. In thirteen of the forty-six hands the two joints were wholly separated by the piso-triquetral capsule. There was no significant difference between right and left hands, and in only three of the twenty-eight subjects were the

joints separate in both hands. The presence of a communication between the piso-triquetral and radio-carpal joints in more than 76% of the fifty-six hands examined, fails to corroborate the textbook statements referred to above. I am indebted to Dr. D. C. Matheson who first called my attention to the condition here described.

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BOOK REVIEW

THE FOETAL CIRCULATION AND CARDIOVASCULAR SYSTEM, AND THE CHANGES THAT THEY UNDERGO AT BIRTH. ALFRED E. BARCLAY, KENNETH J. FRANKLIN AND MARJORIE M. L. PRICHARD; Nuffield Institute for Medical Research, Oxford; Blackwell Scientific Publication, Ltd., Oxford, 1944; pp. xvi and 275, with 160 illustrations, some in color; 50 shillings. Copies may be procured from Charles C. Thomas, 327 East Lawrence Avenue, Springfield, Illinois.

During the winter of 1935 to '36 Sir Joseph Barcroft and his colleagues became interested in closure of the ductus arteriosus at the time of birth. The fetal lamb which they were using for other studies of the blood and circulation proved to be an especially suitable animal for investigating this problem. The next year Donald H. Barron working with Barcroft suggested the x-ray cinematographic technique for observing the course of blood through the fetal heart. Cooperative investigations were arranged with Kenneth J. Franklin and Alfred E. Barclay at the Nuffield Institute for Medical Research, Oxford. From that time until the present a number of reports have appeared. The observations in these have been brought together with much new information to form an extensive consideration of the whole subject of the fetal circulation in the present monograph. Nothing so complete can be found elsewhere. Furthermore, it is well done and quite readable, even in its most technical aspects. The major part of the book was written by Dr. Franklin; the second chapter only, by Dr. Barclay and Miss Prichard. The authors have dedicated the volume to Sir Joseph Barcroft and Dr. Donald H. Barron and have extended acknowledgments to a large number of other people and organizations.

The first chapter of the monograph, Part One, is an historical account divided into several periods. The accounts of the first two, through the time of William Harvey, summarize longer historical articles previously published by Dr. Franklin. Much has been done to clarify certain mis-statements found in the literature. The authors go on in the account of the post-Harveian period to show trends of thought throughout the later years when anatomists tried to deduce from structure alone what the function was. How close some were to the truth and yet how far away were others, could not be fully appreciated until the type of experimentation of the Oxford-Cambridge team made its appearance.

In the second part of the book, Chapter II, the operative procedures and techniques used to obtain x-ray cinematographic records are considered. Care was exercised to assure good physiological conditions in the fetus during experiments. A detailed description is given of the special x-ray photographic technique used to record passage of contrast material through the heart and great vessels. Two types of recordings were used. In one of them a series of x-ray exposures was made on film 5 inches wide at the rate of four frames per second. This method produced the clearest images but another, indirect method was found very useful to obtain records on 16-mm. motion picture film; an image on a fluorescent screen was photographed by means of a very fast lens.

The rest of the second chapter is devoted to a description of the results obtained by these methods—the actual course of the blood flow in the fetal lamb. Beautiful x-ray photographs illustrate successive stages of the passage of radio-opaque material through the vessels and heart in an adult animal (cat) and in the sheep fetus. In some experiments the contrast material reached the heart through the superior vena cava and in others through the umbilical vein, ductus venosus and inferior vena cava. The painstaking and detailed experiments which are described in this chapter of the book cannot be adequately covered in a review as brief as this. One must read the authors' own account of these and examine carefully their illustrations to fully appreciate the splendid results.

To summarize very briefly, they found that radio-opaque material introduced through the superior caval stream passed through the right atrium and the right ventricle of the heart without traversing the foramen ovale and entered the pulmonary trunk. A very appreciable amount went through the pulmonary circulation and the rest of it was shunted by the ductus arteriosus into the descending aorta. Other experiments were performed to identify the ductus arteriosus definitely and to study the changes in the flow from the superior vena cava when the ductus arteriosus closed.

In an equally striking manner the authors demonstrated the course of radio-opaque material traversing the inferior vena cava from the umbilical vein. It was found to pass very largely through the foramen ovale into the left atrium of the heart, the left ventricle and thence through the arch of the aorta and the vessels to the heart itself, the upper extremities and the head. A small fraction of the inferior caval stream traversed the right side of the heart and thence went through the pulmonary circulation and ductus arteriosus.

A most interesting and instructive series of experiments demonstrated the passage of radio-opaque material through the liver of the sheep fetus, and some excellent x-ray photographs illustrate these experiments.

Part Three of the monograph is concerned with an attempt to tell the full anatomical and physiological story of the cardiovascular system and circulation of blood in the mature sheep fetus and the changes which occur during and after birth. Included in this part of the book is a series of accounts of birth of the lamb and rupture of its umbilical cord. Here too is a glossary of new names for certain parts of the fetal cardiovascular system. The authors propose the new terminology on the basis of concepts introduced by the present experimental studies. They have emphasized their recommendations by having the principal terms reprinted on a book-marker which is attached in the binding by a ribbon.

The most notable changes are: sinus intermedius in place of "portal sinus;" crista dividens in place of "annulus fossae ovalis"; via sinistra and via dextra for the two channels into which the inferior caval channel divides within the heart (via sinistra is substituted for the old term "foramen ovale," and for "valve of the foramen ovale" the authors recommend using the phrase, free or appposable portion of via sinistra); crista interveniens for "tubercle of Lower" (the ridge at the union of the via dextra and the superior caval channel); and crista reuniens for the ridge at the union of the ductus arteriosus and the first part of the aorta.

As good as the reasons are for substituting new terms for the old ones which were based on an inadequate understanding of function of structures in the fetal

heart, it is exceedingly doubtful if anatomists and physiologists will readily accept all of them. It will be very hard to get used to calling the foramen ovale the *via sinistra*, though the latter is definitely a more appropriate term.

Part Four deals in two chapters with comparative anatomical and physiological studies. The chapter on comparative anatomy is somewhat lacking in strength and certainly in completeness. A rather curious assortment of fetal animals are portrayed photographically. These include kangaro, hippopotamus, camel, tapir, elephant, whale, bear, lion and gorilla. Many very interesting facts have been brought out by this study and, without doubt when time and material permit, the authors will add to our knowledge of the comparative anatomy of the fetal heart, and the other parts of the cardiovascular system.

The next four chapters of the book, comprising Part Five, deal with the cardiovascular system and course of the blood flow in the human fetus as well as with changes occurring at human birth. In these chapters, the authors have brought together many widely scattered observations and have presented them in a most readable fashion.

Part Six, slightly more than two pages in length, is labeled "concluding remarks" and is somewhat of an anticlimax. An excellent bibliography and an index are appended. It is noteworthy that research and publication on this subject, so unrelated to the war effort, was sponsored at the present time and in a land which has suffered so grievously in the present conflict. No small commendation to those responsible! A word of appreciation is due the publishers, Blackwell Scientific Publications, Ltd., for excellence of the printing and quality of the paper — apparently in no way inferior to that employed before the war.

The *Foetal Circulation* will be read with great interest not only by obstetricians, embryologists and anatomists but by all who want a broader knowledge of fetal and neonatal physiology.

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BOOK REVIEW

SPEED IN ANIMALS, THEIR SPECIALIZATION FOR RUNNING AND LEAPING. By A. BRAZIER HOWELL, University of Chicago Press, Chicago, XII + 270 pages and 55 line drawings, 1944 (\$4.00).

This is one of the monographs sponsored by the University of Chicago Committee on publications in Biology and Medicine. The book is divided into the following ten chapters: (1) Fishes and Amphibians, (2) Reptiles and Birds, (3) Monotremes and Marsupials, (4) Placental Mammals, (5) The Muscular System, (6) The Axial Skeleton, (7) The Arm, (8) The Leg, (9) Proportions, and (10) Gaits. These are followed by 5 pages of summary, by the literature cited, and by a brief index.

In the foreword Professor Howell tells his readers "The locomotion of animals has always been of particular interest to me because of its basic importance in influencing form and function and because it is the dynamic expression of bone and muscle anatomy. I have had this book in mind for many years and during that time I gradually accumulated . . . the information that would enable me to write it . . . In pursuing the subject I have followed the method and inquired into the details that proved to be of greatest interest to me individually. Also, the presentation reflects my own beliefs."

Speed in reaching a distant object may be attained with running, leaping, brachiating, flying, or swimming. It is, however, with only the first two of these means of progression, that is to say, with movement on the ground, that this monograph is concerned.

The author may be said to place before us an unusual and very attractive thesis on the comparative anatomy of the bones and muscles of the trunk and limbs and on gait, particularly on the gait of the race horse, and this he does in a very acceptable manner. Bones and muscles constitute but one aspect of the subject of speed—the joints, such physiological aspects as the neuro-muscular, circulatory, and respiratory responses, and such topics as training and second wind do not form part of the presentation.

There are nine or ten references to man, but these are all very brief, excepting that on the pelvis and that on the controversial subject of the mechanism of the foot in walking which receive a page or so each.

The text bristles with items and observations of general interest. These add greatly to the attractiveness of the work. Some excerpts, taken at random from their setting, may be quoted:

"Tarsius is phenomenally proficient in leaping . . . As in the case of the frog, specialization is for a single sudden, bipedal leap in pursuit of insect prey . . . The natural procedure is for the animal to spring from one upright limb of a tree to another, with astonishing precision and control of balance. At least 8 feet (Lewis, '39) can be covered at a single bound." "I have paced the latter (prairie hare) slightly in excess of 35 miles per hour," "The ostrich . . . can attain 50 miles per hour for $\frac{1}{2}$ a mile, according to the late Martin Johnson (manuscript)." "The running of a lizard may be compared to the operation of an

automobile. The driver may start and stop the car, go fast or slow, and steer, but he cannot change the order of firing of the cylinders; and it is likely that the lizard cannot alter the sequence of limb movement." "Camels progress by the walk, the pace, and the gallop. Under a load the rate is very slow and scarcely exceeds 2 miles per hour. Their swiftest gait is the gallop, but this is evidently very fatiguing and is adopted only for a short dash or under protest. The distance from Suez to Cairo is 84 miles, and there are many authentic statements of camels having covered the distance in 13 hours, 12 hours and less." "The caribou with heavy antlers finds it less fatiguing to trot, so it never gallops for long distances; trotting or pacing would doubtless be very awkward for the giraffe, so it always gallops when in a hurry. Why the opossum never gallops (so far as I know) is problematical." "It has frequently been stated that cursorial habits increase the development of the iliopsoas muscle, probably because this muscle complex is large in the rabbit. Flexure of the vertebral column in the sagittal plane is very helpful to some mammals, such as the weasel, cats, and swift dogs during running; but the propulsive force concerned is furnished by the back muscles in straightening the column, while contraction of the iliopsoas in bending the back and advancing the thigh is merely the recovery action." "As might be expected, high spinous processes over the anterior thorax are characteristic of those animals with extremely large heads or large antlers (Elephas, Brontotherium, Toxodon, Bison). Because of the angle of leverage, this feature is more pronounced in those forms in which the head is habitually held very low (as Bison). Correlated with it are heavy forequarters and light hindquarters . . . One would not expect extreme speed in an animal having this feature in very pronounced degree, but it can whirl about with surprising celerity." "An extremely heavy head is probably not the burden to an animal that one might suppose; for, at least, in many kinds of mammals having this attribute, the ligamentum nuchae is so arranged that its pull practically equalizes the weight of head and neck. This may be demonstrated on a dead ungulate." "A mammal may have a relatively short neck if it is a browser and hence does not need to bring its head to the ground for eating. Among grazers, however, it is the rule that the neck must be as long as the forelegs." "The unguligrade condition is, of course, the highest type of cursorial adaptation; but among living ungulates the hippopotamus and the rhinoceros have not attained it, having a fibrous cushion at the back of the foot that bears the weight of the body, while the camels have secondarily retrogressed in this regard and no longer have hooves."

The author has taken the measurements of the long bones of the limbs of about 300 animals, and in the chapter on proportions he discusses these in relation to speed.

The book will be of particular interest to the advanced student, to the graduate student, and to the teacher. They will read it with enjoyment and profit and will read it again for the many broad principles here involved and for the suggestive and enlightening details presented.

Professor Howell deserves much praise for this clearly and concisely written work on a subject which he presents from a new angle.

J. C. B. GRANT
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BOOK REVIEW

THE PRECENTRAL MOTOR CORTEX. Edited by PAUL C. BUCY. The University of Illinois Press, Urbana. Pp. I-XIV, 1-605. 1944. \$4.50.

This volume on the motor cortex is a monumental gathering of the current views and interpretations of a most difficult subject. A classical paper by Bubnoff and Heidenhain, "On excitatory and inhibitory processes within the motor centers of the brain" has been translated and reprinted; the rest of the chapters have been written especially for this book by individuals well able to review the field. Many papers contain quantities of hitherto unpublished data and opinion. The effect of this volume will be to serve as a cross-section of present neurological thought on the motor cortex, to tighten up thinking, and to entice investigators into the field. Perhaps there is less prospect of the current textbooks being led to abandon their old misinterpretations of the "voluntary motor system." The book is of importance and its effect on current research will be great and beneficial.

The treatment of the subject encompasses all approaches. In addition to the classical authors mentioned, material has been contributed by John Fulton, Paul Bucy, Gerhardt von Bonin, James O'Leary, A. Earl Walker, Sarah Tower, Warren McCulloch, Margaret Kennard, Percival Bailey, Wilbur K. Smith, Theodore Erickson, Charles Aring, Charles Davison, and Marion Hines. Anatomy has fared best, physiology has been adequately treated, clinical symptomatology and pathology have been included.

As is inevitable in such a volume of papers by different workers in a field, quality varies not only with the authors taking part, but with the relation of their chapters to their own program of publication. This peculiarity of *Festschriften* (the volume is dedicated to Otfried Foerster and in a less explicit way to Dusser de Barenne), while leading to certain republication of data already seen by many readers, is offset by an advantage to the reader in many instances where an informality not found in the journals gives an insight into the thinking of the contributors. The first chapter, on architecture, by von Bonin is an illustration. Much new material is given and an important new classification of the subdivisions of Area 4 in the human cortex is presented. The reader will better be able to know the author through reading his speculations on the physiological implications of the morphology of the pyramidal cells. Some of these approach the limits of scientific usefulness — as, for example, the guesses concerning the functions of basal vs. apical dendrites.

Of particular interest is the discussion of the problem of interpretation of electrical activity of the cerebral cortex by James O'Leary. Careful and well-considered, it could have profitably digressed more on the use of the time factor as a device for analyzing neuron systems. The correlation of histological studies, particularly the results of Golgi the method of staining, are properly stressed. As this difficult approach has not yet yielded information on the motor cortex, the inclusion of this chapter reflects the editor's hopes that knowledge of the motor

areas will soon be augmented through investigations utilizing the cathode-ray oscillograph.

Bucy's chapter on extirpations in man adds considerably to the available information on this important phase of the subject. It is the most elaborate discussion to date and contains new material of great value.

The chapter on the pyramidal tract by Sarah Tower is outstanding as a careful summary of the anatomy and physiology of the cortico-spinal tract. It is an eloquent testimony to the value of animal experimentation, even in investigation of a so characteristically human structure as is the cerebral cortex. The chapter consists of interpretation of her previous papers, especially that on pyramidal lesions in the monkey, though new material on experiments performed on the chimpanzee is discussed. Many readers will wish to reread the protocols of her 1940 paper concomitant to study of this chapter. Of especial significance is her conclusion that the most characteristic deficit produced by pyramidal lesion is loss of fine control of movement. Clinical diagnosis and interpretation would profit by increased emphasis upon this for the "cortical signs" so widely used have been proved much less reliable than previously supposed.

Without reservation, the various chapters not mentioned specifically are recommended as important summaries and reinterpretations of old and new findings. To discuss all of them individually would be impossible here. Especial attention is due, however, to the concluding chapter by Marion Hines. This consists in part of a careful evaluation of the methods available for study of the motor cortex. Her attitude is perhaps best described as constructive scepticism. Critical appraisal is directed in turn at descriptions of deficits and symptoms, the architectural studies of the motor cortex, the records of human stimulation, and the interpretation of ablation experiments. The abominable verbal difficulties of this field lead her to preface the discussion of these subjects with the following statement. "This appraisal of the contribution made to the control of movement by the precentral motor cortex will be attempted with as little use of customary terminology as possible." Nor does the clinical pedagogue escape! "The use of the term 'upper motor neuron lesion,' as a contrast to 'lower neuron lesion,' has caused many to attribute the sequelae of damage to the cerebral motor systems to injury of a single corticofugal system, the pyramidal tract. Such thinking denies the participation in the results of such lesions to other known corticofugal systems." Also contained in this chapter is an exposition of Dr. Hines' ontogenetic approach to the problem of motor cortex physiology.

The book concludes with a long and useful bibliography and is well indexed. The figures and printing are of high quality. The publishers, with the editor and contributors, are to be congratulated on their excellent and important contribution to neurology.

BERRY CAMPBELL
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EDITORIAL

Beginning with this issue, the Editorial Board in conjunction with the Committee on Nomenclature of the American Association of Anatomists (Dr. George W. Corner, Chairman) is presenting a series of articles by seven of the Stanford group of anatomists, dealing with the present unsatisfactory status of anatomical nomenclature. This series is an outgrowth of their weekly discussions of the problem, carried on throughout the past year. In this interval, as Dr. Danforth points out, there has been revealed such "diversity of opinion as to make preliminary discussion desirable before any committee of the Association is called upon to formulate a statement which can be passed on to our colleagues in other countries."

After this series of articles has been published, or coincident with them, it is proposed to invite the British Committee and our Canadian Colleagues to state their views, following which discussion will be opened to members of the Association, or to others who wish to put specific questions and proposals.

While the primary function of *The Anatomical Record* is the publication of original research, it is felt that no more favorable time could be found for a reconsideration of nomenclature than under the present war conditions when more space is temporarily available, when post war conditions have not crystallized and when the accelerated teaching program has focused attention on the question of nomenclature. The following statement from Dr. Corner presents the official status of the problem:

"By emphasizing, at this critical time, the dangerous confusion in anatomical terminology, the Stanford University Anatomists have rendered a great service to their colleagues. As Chairman of the official Committee on Nomenclature of the American Association of Anatomists, and the only member still in active service of the group, which wrestled with the problem just before World War II, I share their feeling that we must take prompt thought on the subject if the confusion is not to get much worse. To this end I offer, as a contribution to the proposed series of comments, a statement of the official activities of the American anatomists with respect to the nomenclature problem.

"The history of the original formulation of the BNA and its adoption in the U. S. need not be recounted here, nor the story of its use until the 1930's, when movements for revision began in several countries.

"The outbreak of war in 1939 put an end to international scientific cooperation at a most unfortunate time as regards anatomical nomenclature, for just at that

period anatomists of the U. S. were faced with two conflicting attempts to revise the terminology and were working toward an international solution of the problem. In 1933 the Anatomical Society of Great Britain and Ireland published the British Revision (BR) and in 1936-37 the Anatomische Gesellschaft brought out *Nomina Anatomica* (NA), a revision of the BNA. The American Association of Anatomists, requested by our British colleagues to join them in presenting the BR to an international group, took the more cautious step of appointing a committee of its own. Our committee (C. M. Jackson, Chairman, the late S. W. Ranson, R. J. Terry, the late T. W. Todd, and G. W. Corner) studied both the proposals with great care from 1933 to 1937. It believed that American anatomists would not accept the British vernacular nomenclature (BR) largely (as I shall discuss below) because the British insisted on the old system of indicating direction and relative position. The Americans felt that the BNA had served very well, meeting the needs of modern anatomical sciences in the broad sense, including human embryology and the comparative aspects of gross human anatomy, better than the proposed British system. The American report took the form therefore of a painstaking and rather moderate revision of the BNA, drawn up in Latin like the original. It was forwarded to the International Commission on Anatomical Nomenclature, created by the International Congress of Anatomists at Milan in 1936. The whole subject was to have been dealt with at an international congress in London late in 1939.

"The American Committee never published its report, not wishing merely to set up a third terminology to compete with the BR and NA. All it hoped for (in vain, alas) was to get its views before the anatomical world, and to be prepared as fully as possible to cope with the rising tide of nationalism, exemplified by the adoption of the BR in one country and the NA in the other. The American report exists only as a manuscript of 57 pages, of which there were no more than a dozen copies, one of which is presumably in the files of Professor Stieve, secretary of the International Commission.

"In order to keep the American anatomists in readiness for action when the time comes, the American Association in 1942 appointed a new committee consisting of E. A. Boyden, Sam Clark, C. H. Danforth, W. W. Greulich, and George W. Corner, Chairman. There has been no occasion for action by this committee.

"The problem of anatomical nomenclature breaks down into two major divisions. In the first place, there is the ordinary question of the best name for a given structure (e.g., *submaxillary gland*, BNA vs. *submandibular*, BR, NA). Such questions of course often involve different interpretations of fact, or different ideas about grammar and philology (e.g., *anulus*, NA vs. *annulus*, BNA) or more competing national customs. They can be troublesome, especially if it is hoped ever to get the French, Italian, Portuguese, Russian and Spanish-speaking anatomists to join in a really international terminology; but these problems can after all be worked out by conference and compromise. Many of the reforms proposed by the BR and the NA groups were acceptable to each other and to the Jackson Committee.

"The real difficulty has to do with the terms describing relative position, for example *dorsal* vs. *posterior*, *upper* vs. *cranial*, etc., etc. It may surprise many younger American anatomists to know that the American Society of Great Britain and Ireland officially discards the terms *ventral* and *dorsal* to denote relative position in the human body. The BR considers only man in the "anatomical position" and speaks of *superior* and *inferior*, *anterior* and *posterior*. The NA, on the other hand, in its anxiety to create a terminology suitable for all branches

of human anatomy, went to extreme lengths to set up terms that would apply no matter what the position of the body, for example speaking of *cranial* and *caudal mesenteric arteries*. The same vessels in the horse, incidentally, are called by veterinarians *anterior* and *posterior* mesenterics.

"These terms of relative position and direction amount to about 500 of the 5000 terms in the BNA. There was (and still is) no hope of getting the British to accept the system which the Germans and Americans had found acceptable, nor on the contrary of getting our own people to discard such terms as *dorsal* and *ventral*. This same difference is the major obstacle to participation of the French-Italian-Iberian-Latin American groups in an international terminology.

"We thought it a great pity to lose the chance of agreement on all the other questions of a 5000-word terminology for the sake of the 500 terms involving direction and relative position. We proposed therefore to allow both usages, thus

Rami dorsales (= posteriores)

The British could go on saying *posterior* and we could say *dorsal*, and so on for *ventral* = *anterior*, *superior* = *cranial*, etc. The acceptance of such a compromise might be all the easier because of the fact that the British were primarily interested in an English-language terminology, whereas we were aiming at an international Latin nomenclature upon which the various nationals might base their vernacular discussions in hospital and dissecting room.

"We had high hopes that at the international conference some such compromise would permit a serious effort to get agreement on the real scientific and philological problems of terminology.

"This is where the matter now stands. The American anatomists, tacitly and by advice of their Committee, have gone on using the original BNA; the German and Japanese societies adopted the NA; the British are using the BR and it is drifting into our own books; the French and all the other Romance-language anatomists are using the terminology of Testut and Poirier and Charpy. Thus there are four terminologies in use, or to be used again when science revives on the continent of Europe. If Dr. Jackson's committee had not been cautious (or quixotic) about publishing its own report of 1937, there would be five. We are losing all that was gained by the toil of our predecessors.

"The time is coming rapidly when American anatomists will have to take an official stand on this difficult matter. To maintain our own broad scientific principles; to meet our British colleagues, more than ever bound to us by ties of sacrifice and mutual interest, in a cooperative spirit; to bring our science closer to that of the Latin Americans who are working with us on a thousand problems of medical science; meanwhile not to lose touch with the old sound European scholarship, which will revive again, even in the Germanic lands; these are the tasks which face us if we want an international terminology for the one thing common to all mankind, the human body."

It is requested that all contributions dealing with the subject of nomenclature be sent first to the office of the Managing Editor. Since there will be instances in which the opinions proffered could be illuminated by the experience of the older committee and its successor, manuscripts will also be cleared through these channels. Therefore they should be submitted with an attached carbon copy.

E. A. BOYDEN,
Managing Editor

SHOULD THE BNA BE ABOLISHED?

Anatomists have long desired a stable nomenclature, international in scope and freed from sectionalism and burdensome synonymy. A significant expression of this desire was the adoption of the Basle *Nomina Anatomica* to which most anatomists, occasionally submerging personal preferences, gave whole-hearted support. It was at first hoped that after the BNA had been taught for a few years to a succession of beginning students, the terms would become thoroughly entrenched among the younger anatomists and that their use would automatically spread to the other medical disciplines. To a certain extent this has indeed happened; but the progress, while perhaps steady, has been less rapid than might have been expected. During the last few years, however, further progress has been seriously jeopardized by a lack of conformity on the part of authors and publishers that threatens to undo much of what has already been accomplished. The situation is one that justifies consideration by anyone at all interested in anatomical terminology.

It seems to have been clearly foreseen when the BNA was adopted that defects would be found, and it was expected that essential revisions would of necessity be undertaken from time to time and submitted to anatomical congresses for ratification. Several national committees, especially those of the British, German, and Japanese associations, have drawn up proposed revisions which in ordinary times would have been presented to an international congress for consideration and proper action. But with normal discussion of these proposals made impossible for the time being, there has appeared of late an impatient tendency to advance various of the suggestions as if they had already been adopted, and were therefore authentic. Not all of the proposals have been formally presented to the American anatomists, and they are not all consistent with one another, but some of the proponents of these "revisions" are insisting upon them almost to the point of an avowed break with the BNA code, something which must produce an unfavorable effect on our students and on our colleagues in other fields.

The condition has seemed so serious that members of the Department of Anatomy at Stanford University have been holding regular meetings to discuss this subject and clarify thinking in regard to it. In these conferences we have had the valuable assistance of Raymond D. Harri-

man, Professor of Classics, who has given us the benefit of his knowledge and interests in the field of scientific terminology. Enough diversity of opinion and attitude has been revealed among ourselves to suggest that American anatomists in general may not be in entire agreement in regard to problems of anatomical nomenclature, and that some informal preliminary discussion may be desirable before any committee of the Association is called upon to formulate a statement which can be passed on to our colleagues in other countries. No system of terminology is likely to succeed if it does not have the support of those by whom it is expected to be used. It was hoped that the subject would be brought up at the Cleveland meetings, but since those meetings have now been cancelled, we have ventured to ask *The Anatomical Record* if some space may be given in its pages to the presentation of such views and suggestions along these lines as may be pertinent, in case other anatomists think the matter as important as it seems to us.

There are many terminological issues on which some general agreement or understanding should be reached before even minor revisions can wisely be suggested. For example, it should be agreed whether the selection of general terms is to be based on historical and vernacular implications or on arbitrarily assigned, special anatomical connotation. Should there be a further standardization of noun forms and the types of adjectives derivable from them? Do we want a greater standardization in the use and precise meaning of suffixes? Should embryological and histological terms be added to a revised or rejuvenated code? There are many such questions on which we have found some differences of opinion among ourselves, but it may well be that there are simple answers which will have a universal appeal. In any event, it appears to us desirable that future committees on nomenclature be charged with the responsibility of defining, in so far as may be necessary, the principles underlying the nomenclatorial system to be advocated, including statements on such items as the formation and use of combining forms, derivatives and suffixes.

The only definitive suggestion that we wish to make in this first note, is in reference to the current status of the BNA. Members of the Stanford group are unanimous in feeling that in the interest of a sound nomenclatorial policy each BNA term must be recognized, and insisted upon, as the only official name for the structure or relation which it designates until that term shall have been replaced through regular action of properly constituted representatives of the anatomical associations. Any other policy endangers the gains of the last half century. It is believed that the growing custom of scattering proposed new terms through our textbooks—however desirable these terms may be intrinsically, and even though they are inclosed in brackets—can serve only to

introduce undesirable confusion. It is our feeling that strong representation should be made to editors and publishers with the hope of discouraging this unfortunate practice. Such a stand would have no relation to the question of what future revisions may be desirable. The immediate obligation is to avert the chaos which is threatened by an impending breakdown in our allegiance to an internationally sponsored and approved system of nomenclature.

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R. L. BACON

C. H. DANFORTH

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W. W. GREULICH

H. KIRKMAN

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OSSIFICATION AT THE PROXIMAL TIBIAL EPIPHYSIS IN THE RAT

I. CHANGES IN FEMALES WITH INCREASING AGE ¹

HERMANN BECKS, MIRIAM E. SIMPSON AND HERBERT M. EVANS

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THREE PLATES (EIGHTEEN FIGURES)

The process of endochondral ossification in the rat has been described carefully by several workers (Watt, '28; Dawson, '29; Dodds, '30, '32; Dodds and Cameron, '34; Bloom and Bloom, '40; Ross and McLean, '40; Ingalls, '41). The earlier studies were based on decalcified bones sectioned and stained by ordinary methods, but in later studies, silver stains were employed to show distribution of the calcium. These studies have included analyses of the process of ossification in the very young rat, embryonic and newborn (Bloom and Bloom) and in the very old rat (Dawson). The reports are quite consistent and it might not be evident at first glance why the subject is re-opened here. The answer is to be found in the need for precise information as to the state of ossification of the tibia in normal rats at different ages. This bone is used extensively to study growth rates under experimental conditions. The immediate purpose here is to provide standards for comparison with the conditions found in rats hypophysectomized at an early age and maintained for various postoperative periods (Section II).

Normal female rats of the Long-Evans strain, reared on an adequate diet ² were sacrificed at intervals between the ages of 5 and 546 days. The proximal end of the tibia was decalcified, sectioned, stained with hematoxylin and eosin, and examined microscopically. This region was chosen as typical for a long bone in which the epiphysis remains open for a large part of the rat's life. (Dawson finds that the proximal epiphysis of the tibia closes by the 1135th day of life).

¹ Aided by grants from the Research Board of the University of California, The Rockefeller Foundation, New York City, The American Foundation for Dental Science and the California State Dental Association.

² Diet XIV, supplemented by lettuce. (The diet consists of 5% casein, 68.5% wheat, 10% fish meal, 10% alfalfa, 1.5% NaCl and 5% fish oil).

Figure 1 shows a section of the head of the tibia of a 5-day-old rat. The head of the bone is still composed only of cartilage, chiefly of the embryonic type. Nearer the diaphysis the cartilage cells show some flattening and arrangement which may be called a foreshadowing of column formation. The flattened cells stain deeply with hematoxylin; the area of increased basophilia can be distinguished in figure 2 taken at a lower power. The cartilage cells next the diaphysis (about 7 cell layers) are enlarged and light staining. They are not yet clearly arranged in rows. Capillary erosion of the last layer of enlarged cells is going on vigorously; the trabeculae of the primary spongiosa are delicate and close together.

Figure 3 shows the proximal end of the tibia of a 15-day-old rat. The epiphyseal center of ossification has now appeared (see also fig. 4 taken at lower power) and the embryonic hyaline cartilage has been greatly reduced by the growth of the epiphyseal bone. The epiphyseal cartilage plate which is now being formed³ is composed largely of differentiated cells which are flattened and are arranged in fairly well-defined columns. They are larger than the embryonic cartilage cells and have deep staining nuclei which give the region a predominantly basophilic stain. They are still separated by relatively large amounts of matrix. Next the diaphysis the cells in the last six or seven layers are much enlarged and their nuclei and cytoplasm have both become lighter staining. The last row or two show the greatest amount of vacuolation and the nuclei have completely disappeared from some of these cells.

Figure 5 shows the region of the proximal epiphysis of the tibia of a 20-day-old rat. The epiphyseal cartilage plate is now clearly defined due to expansion of the epiphyseal center of ossification (see also fig. 6). The embryonic cartilage, next the epiphysis, is represented by only a few cells, most of these cells having increased in size and become marshalled into rows. The zone of flattened basophilic cells is now wider, occupying approximately half the width of the disc. The parallel columns of flattened cells are now clearly continuous with the columns of enlarged cells. There are about four to five layers of enlarged cells next the diaphysis; all stain lightly, both cytoplasm and nucleus. Paralleling the growth of cells during this period there has been a relative decrease in amount of matrix so that by 20 days it is represented merely by a thin layer between columns. The bony trabeculae are delicate and closely packed as can be seen in figure 6.

³ The epiphyseal center of ossification had already appeared in two of three animals 10 days of age. Vacuolated cartilage cells were present in the head of the shaft in the third rat. (Not illustrated.)

Figure 7 shows this region in a rat of 26 days of age. The epiphyseal cartilage is still about the same width as at 20 days. It can be divided into three zones: (1) a narrow zone of irregularly arranged embryonic cartilage cells next the bony epiphysis. The cells are small, have very little cytoplasm, and are scattered sparsely in an ample hyaline matrix. (2) The next zone consists of columnarly arranged cells occupying over half the width of the cartilage. These cells are definitely larger than the embryonic cells and are flattened transversely in the rows. The nuclei stain deeply with hematoxylin. The cell columns are separated by narrow strips of basophilic matrix, but practically no matrix occurs between the flattened cells within the columns. This zone may be called the basophilic zone because of the depth of the hematoxylin stain, or simply the zone of flattened cells.⁴ (3) In the half of the cartilage disc adjacent to the diaphysis, the columnarly arranged cells become enlarged and change shape. The flattened cells first become rounder or polygonal and finally may become elongated in the direction of the columns. Even less matrix is present in this zone. The cells are vacuolated and the nuclei take little hematoxylin, hence the pale stain in this area. This zone may be called the vesicular zone or simply the zone of enlarged cells. Capillaries at the line of erosion are numerous and are directed against each cartilage cell column. The cartilage cells are being removed with great regularity, giving a straight, clearly defined margin. The inter-columnar matrix remains to form the core of each delicate spicule of newly formed bone.

This is the condition of the epiphysis at the age at hypophysectomy (26-28 days) of the rats described in Section II of this paper, and figure 7 will be used as the standard of reference in discussing the changes which occur following the operation.

Figure 8 illustrates the condition at the proximal epiphysis at 30 days of age. The cartilage is still wide. The vesicular zone occupies about one third the width of the cartilage; the layer next the marrow is differentiated by extensive vacuole formation.

Figure 9 shows the condition at 42 days of age. The cartilage is not significantly reduced in width.⁵ Some reduction in cell size has now occurred within the basophilic zone. The vesicular zone has decreased

⁴ Sometimes during preparation of the sections the tissues are shrunken, in which case this zone shows the change conspicuously; the somewhat flattened cells in the columns become more flattened and the matrix takes a very deep basophilic stain. This may cause a very sharp line of demarcation to appear between the basophilic zone and the next zone of enlarged cells.

⁵ The width in micra of the epiphyseal cartilage in animals of different ages may be seen in Section II of this paper, table 1.

slightly in width and is now composed of only three to four cell layers. The cells are not highly vacuolated except in the layer next the marrow. Capillary invasion still seems to be directed against each of these vacuolated cells which terminate the cartilage columns, so that the primary trabeculae are still close together.

Figure 10 illustrates the condition in the epiphyseal region in a 56-day-old rat. The cartilage is now definitely narrower. A slight increase in matrix can be seen in the zone of flattened cells, accompanied by some decrease in the number of cell layers. The vesicular zone is narrower and vacuolation of cells in the layer next the marrow is now intermittent. A slight decrease in the number of capillaries in the line of erosion is just perceptible and the trabeculae may be somewhat coarser.

Figure 11 shows a section from a 74-day-old rat. The cartilage plate has undergone a marked reduction in width, which can be related to the decreased growth rate characterizing the female rat of this age. Only a few unoriented embryonic hyaline cartilage cells are still seen next the epiphyseal bone. The diminished width of the cartilage is due to decrease both in cell size and number of cell layers. In the zone of flattened cells the reduction in cell size is more conspicuous. In the vesicular zone there has been marked reduction in numbers of cell layers, only three to five layers now being present whereas at 26 days there were six to eight layers. Cell size has also been reduced in this zone, and a polygonal shape is now characteristic, rather than the elongation in the direction of the columns which characterized the more rapidly growing phase. The intercellular matrix is definitely increased, more markedly so in the zone of flattened cells towards the epiphyseal bone plate. Capillaries are apparently no longer attacking each individual cell column, at least trabeculae of the primary spongiosa are no longer found continuous with individual spicules of intercartilaginous matrix. Bony trabeculae are coarse, even immediately adjacent to the cartilage, and become rapidly coarser as they extend into the marrow. These trabeculae still are composed almost purely of lamellar bone so that resorption of cartilage and true endosteal osteogenesis are both still vigorous. The trabeculae have lateral anastomoses and are quite closely packed. Alternating with the diaphyseal bony trabeculae, areas of cellular marrow now occur.

Figure 12 shows the condition at 123 days of age. There has been a marked reduction in width of the cartilage corresponding to the beginning of the growth "plateau" in the female rat. The number of flattened cells now present in the columns of the basophilic zone is re-

duced, varying from four to twelve cells. Where only a few cells are present in the columns the intervening space is occupied by matrix. In some places two or more columns are grouped together and are set apart by masses of matrix, so that their arrangement already begins to suggest the "conical" pattern seen in old rats by Dawson ('29). Due to all these factors the columns are no longer so strictly regular and parallel. The vesicular zone consists of only two to three cell layers and the cells are no longer as markedly enlarged. Some calcification of the matrix throughout the vesicular zone is suggested by the increased basophilia.⁶ The amount of cartilage matrix incorporated in the bony trabeculae has increased, fused cartilage matrix, corresponding in width to two or three cartilage columns, being now continuous in places with bony trabeculae. The trabeculae are still covered by a thick layer of bone, so that the composition of the trabeculae has not essentially changed; bone still dominates over cartilage matrix. This continuity of cartilage matrix with bone obliterates the sharp line of demarcation which originally existed between cartilage and marrow. Capillaries can still be seen in places to be invading individual cartilage cell columns, but this condition is now the exception. The trabeculae of bone are not only coarser, but also farther apart than at 74 days; lateral anastomoses are more conspicuous, so that the orientation of the trabeculae parallel to the long axis of the bone is beginning to be obscured.

Figure 13 shows the status at 170 days of age. Very little further atrophy has occurred in the cartilage. Masses of cartilage matrix, sometimes including cells, are continuous with and form the cores of the coarse trabeculae. The only capillaries which still establish contact with cartilage may now be described as occurring in islands in the mesh of trabeculae. The cartilage between trabeculae is now largely sealed from the marrow by calcification of the marginal cartilage.

Figure 14 shows the cartilage and trabecular bone at 251 days of age. The cartilage has now been reduced almost to the minimum width observed in this series. It is quite irregular in outline due both to the indentations made by epiphyseal lamina and to continuity of its matrix with diaphyseal trabeculae. The amount of cartilage extending into the trabeculae has greatly increased since 170 days of age and the cartilage cores extend much farther into the shaft. This enclosed cartilage (also the marginal cartilage) shows a new phenomenon. The

⁶ Calcification of this zone, so that it forms a continuous band across the cartilage, does not occur in the normal rat as it does characteristically in the hypophysectomized rat after long postoperative intervals.

✓✓

matrix surrounding the cartilage cells stains like bone, forming globular masses which are embedded in unchanged, non-cellular matrix. The surfaces of the trabeculae are covered with true bone, but the proportion of true bone in the trabeculae has been greatly reduced. The "sealing off" of the cartilage from the marrow by continuity of the cartilage with these trabeculae, and by calcification of marginal cartilage matrix, is now well advanced. Cartilage cells are sometimes enclosed in the calcification of this marginal cartilage.

Figure 15 shows the region of the epiphysis in a rat 307 days old. This illustration shows well the density of the lamellar bone of the epiphysis, also the presence of the lamellar bone on the surface of the trabeculae of the diaphyseal bone.

Figure 16 shows the condition at 384 days of age. The cartilage is thin and irregular in width but is still continuous across the disc. The cells are all small and matrix is abundant. Very little enlargement of cartilage cells and no vacuolation is seen, and the columnar arrangement is almost lost. A vesicular zone cannot be said to exist. The cartilage cells must, however, still be multiplying as shown by the number enclosed in the masses of matrix within the trabeculae. The only enlarged cartilage cells are seen here. The calcification of the capsule around the enclosed cartilage cells is well shown in this section. The marginal cartilage is almost completely calcified. Only in isolated areas do the capillaries still attain contact with cartilage; no response of the cartilage appears to be associated with the contact, as the cells neither show orientation to the capillaries nor are they enlarged or vacuolated.

Figure 17 shows the region at 502 days of age. Very little further change has occurred, except that proliferation of cartilage has slowed, judged by the amount of enclosed cartilage in trabeculae. The illustration shows clearly the lamellar bone present, both on the surface of the calcified (metaplastic) cartilage at the interface between cartilage and marrow, and on the surface of the trabeculae.

Figure 18 shows the condition of the epiphysis at the oldest age examined, 546 days. It will be noted that in certain places the matrix of the cartilage stains deeply throughout its width, supposedly due to calcification. Cartilage cells within the disc occur in well-defined conical masses, separated by large amounts of matrix. The area occupied by cells is now less than the area occupied by the non-cellular matrix. The sparsity of trabeculae characteristic of older animals is clearly shown in the illustration. It should also be noted that these trabeculae contain less cartilage matrix.

SUMMARY

The process of endochondral ossification at the proximal end of the tibia has been described and illustrated for normal female rats ranging in age from 5 to 546 days.

The epiphyseal plate forms between 5 and 15 days of age, due to the appearance and expansion of the epiphyseal center of ossification. As was already known, the epiphyseal plate does not disappear until far beyond the maximum age observed here (546 days); however, marked changes occur and these are associated with progressively decreased activity of this region.

1. Changes in the epiphyseal cartilage

The cartilage decreases in width, quite abruptly at the time body growth rate slows, between 74 and 123 days of age, more slowly thereafter. The decrease in width of the cartilage is due to decreased number and size of cells. Columnar orientation of the cells and flattening of the cells within the columns (the latter probably indicating cell division) still persist to some extent to the end of the period investigated, but enlargement and vacuolation of the cells almost cease. Accompanying the decrease in cell size there is an increase in amount of matrix. The cartilage cells become grouped in conical masses (the bases of the cones being directed toward the diaphysis) and the matrix occurs in masses of increasing size between these cartilage cell groups.

2. Changes in the bony trabeculae

In the early phases of growth, capillary loops attack each column of cartilage cells and remove the terminal, degenerating cells; the thin strands of matrix between columns remain as the cores of the delicate bony trabeculae. The trabeculae of this primary spongiosa are numerous, close together and parallel with the long axis of the bone. With increasing age, the trabeculae become coarser and more irregular in shape and their longitudinal orientation becomes obscured. These coarse trabeculae are continuous with and enclose cartilage masses of increased size, chiefly matrix but also some cells. The capsular material of the cartilage cells within the trabeculae may be calcified, giving globular masses. This extension of the cartilage into the cores of the trabeculae destroys the abruptness which earlier characterized the line of demarcation of the cartilage plate. With advanced age the amount of enclosed cartilage within trabeculae decreases, probably indicating a further slowing of cartilage growth. The trabeculae themselves are greatly reduced in number.

3. "Sealing off" of the cartilage

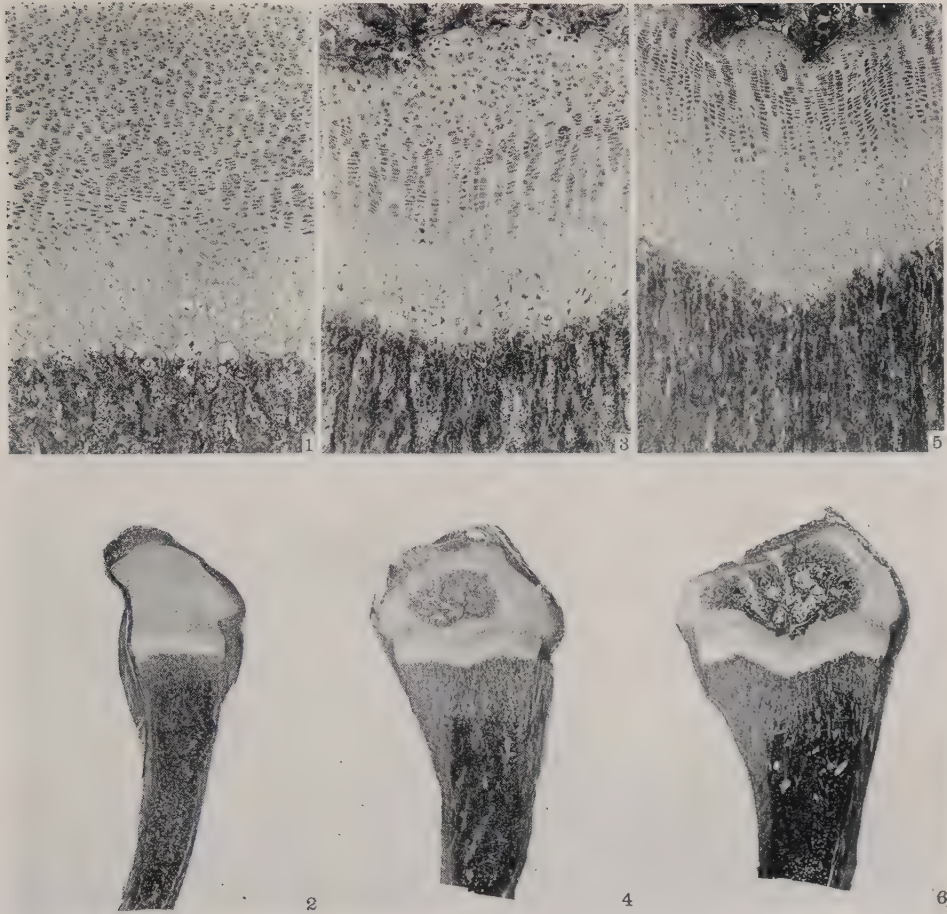
Though the cartilage plate persists in the rat until old age, it becomes almost completely sealed off from the marrow, and so from encroachment of blood vessels. This begins relatively early, at approximately 170 days of age, at which time growth has practically ceased. There are two phases to this process. The first of these is the one just described, in which groups of cartilage cells become continuous with and are incorporated within the trabeculae. The second is the calcification of the marginal cartilage matrix, and cellular capsules. True lamellar bone is laid down upon this transformed cartilage as it is upon the surface of the trabeculae.

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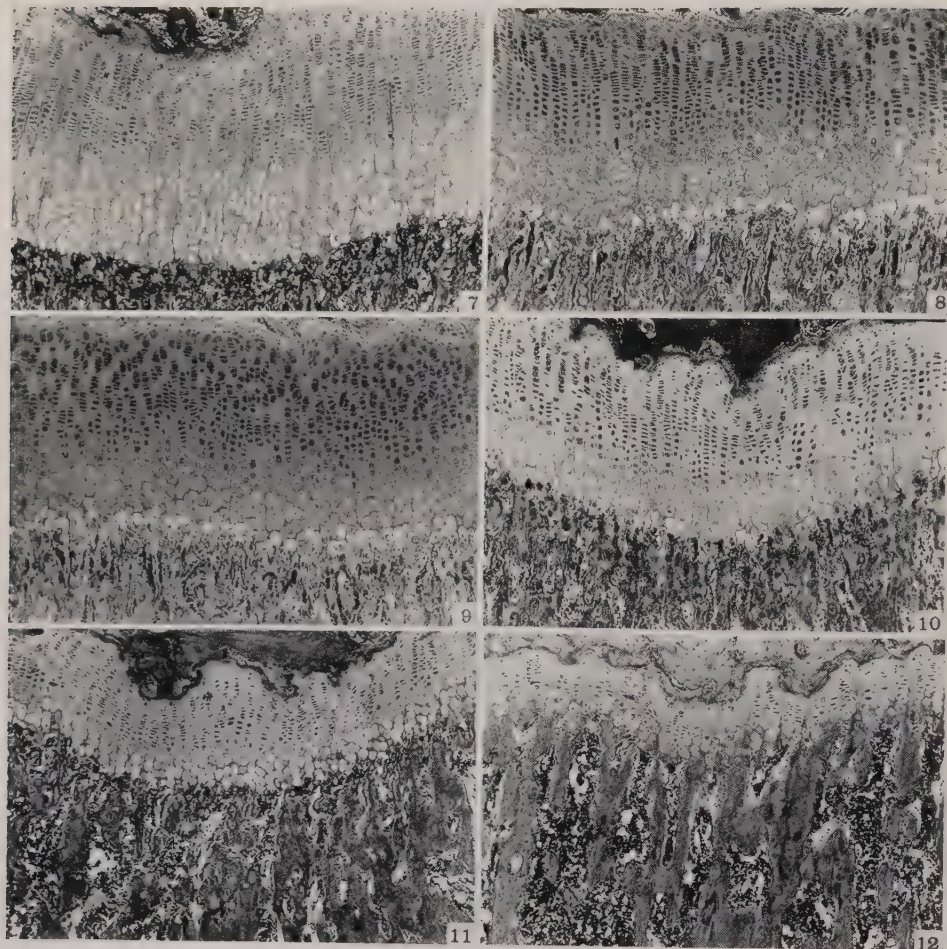
EXPLANATION OF PLATES

Proximal epiphyseal region of the tibia of normal female rats of various ages. H and E stain, $\times 56$ except as otherwise noted.



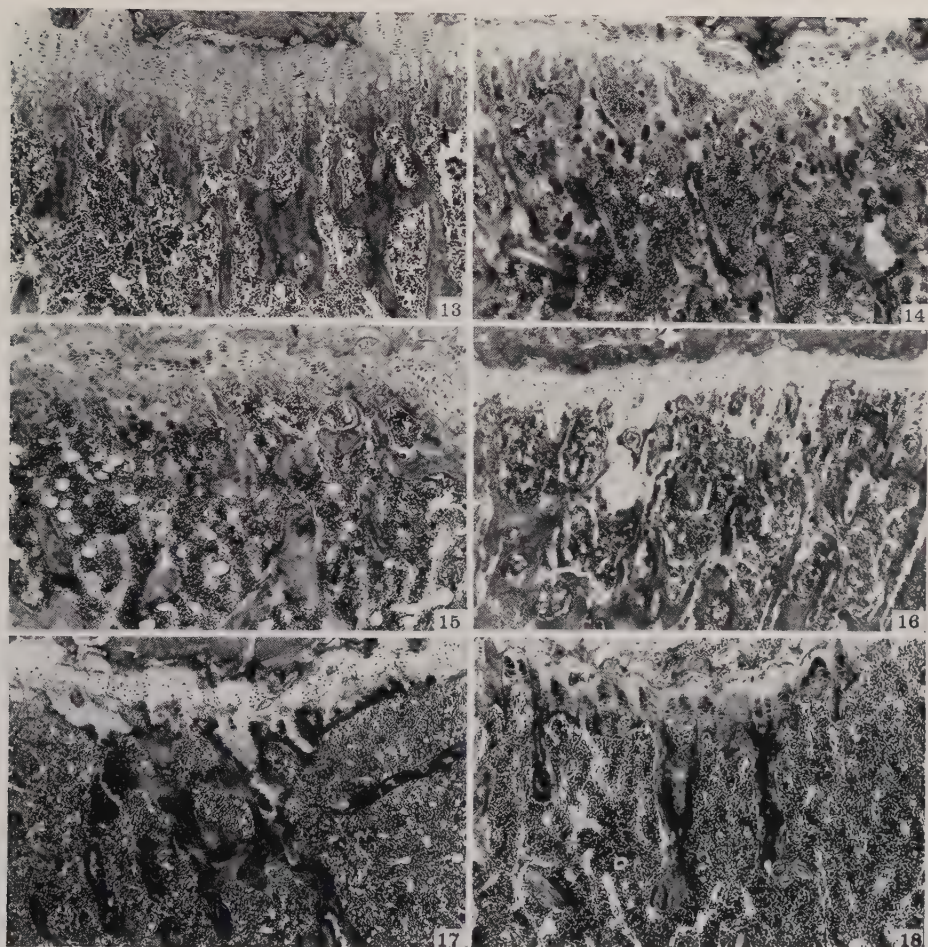
EXPLANATION OF FIGURES

1	Age	5 days.	Sp. 8701.	Pl. 8502
2	Age	5 days.	Sp. 8701.	Pl. 8499 ($\times 9$)
3	Age	15 days.	Sp. 8704.	Pl. 8501
4	Age	15 days.	Sp. 8704.	Pl. 8498 ($\times 9$)
5	Age	20 days.	Sp. 8707.	Pl. 8500
6	Age	20 days.	Sp. 8707.	Pl. 8497 ($\times 9$)



EXPLANATION OF FIGURES

7	Age 26 days.	Sp. 6159.	Pl. 8485
8	Age 30 days.	Sp. 6757.	Pl. 8469
9	Age 42 days.	Sp. 6738A.	Pl. 8470
10	Age 56 days.	Sp. 8368.	Pl. 8500A
11	Age 74 days.	Sp. 6161.	Pl. 8472
12	Age 123 days.	Sp. 6166.	Pl. 8473



EXPLANATION OF FIGURES

13	Age 170 days.	Sp. 6167.	Pl. 8474
14	Age 251 days.	Sp. 8378.	Pl. 8475
15	Age 307 days.	Sp. 8383.	Pl. 8476
16	Age 384 days.	Sp. 6051.	Pl. 8477
17	Age 502 days.	Sp. 8365.	Pl. 8478
18	Age 546 days.	Sp. 6769.	Pl. 8479

OSSIFICATION AT THE PROXIMAL TIBIAL EPIPHYSIS IN THE RAT

II. CHANGES IN FEMALES AT PROGRESSIVELY LONGER INTERVALS FOLLOWING HYPOPHYSECTOMY ¹

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THREE PLATES (FIFTEEN FIGURES)

A number of accounts have appeared in the literature on the effects of hypophysectomy on endochondral ossification in the rat. The essential features, at least in early periods after operation, are given in the studies of Freud, Levie and Kroon, '39; van Eck and Freud, '41; Ray, Evans and Becks, '41, '42; Kibrick, Becks, Marx and Evans, '41; Becks, Kibrick, Marx and Evans, '41; Ingalls and Hayes, '41, and Valle, '44. An account is given here of the changes occurring at progressively longer postoperative intervals in the region of the proximal epiphysis of the tibia of female rats which had been hypophysectomized at a constant age (26-28 days). Of particular interest are the late changes (after 1 to 2 years) and the comparison of the changes with those occurring in the normal rat with increasing age (Section I).

The rats used were of the Long-Evans strain. After hypophysectomy, the diet given normal rats was supplemented daily by a wet mash of a diet slightly modified from McCollum's Diet I.² The operation was performed, as is usual, by the parapharyngeal approach. Only those animals are discussed in this paper in which the characteristics of hypophysectomized rats were already obvious during life, and in which the completeness of the operation was confirmed at autopsy by the finding of a sella turcica free of pituitary fragments.

¹ Aided by grants from the Research Board of the University of California, The Rockefeller Foundation, New York City, The American Foundation for Dental Science and the California State Dental Association.

² The diet consists of 67.5% wheat, 15% casein, 10% whole milk powder, 1% NaCl, 1.5% CaCl₂, 5% homogenized vegetable oil, and a concentrate of fish oil in amount to give 1.52 chick units of vitamin D and 11.4 USP units of vitamin A per gram of diet.

Reference should be made to Section I for a description of the proximal epiphyseal region of the tibia of normal rats of the age at hypophysectomy (see fig. 7 of that section).

Figure 1 shows a photomicrograph made of the region of the proximal epiphysis of the tibia of a rat 4 days after hypophysectomy. The epiphyseal cartilage is already narrower than at the time of operation, being roughly comparable in width to that of a 65-day-old normal rat. The zone of flattened basophilic cells is much narrower than at operation, due chiefly to reduction in cell size. The vesicular zone is disproportionately reduced, the reduction being due to decrease both in cell size and in number of cell layers. There are only about three to four layers of enlarged cells, including the last layer of degenerating cells. Capillaries are still directed against these degenerating cells, but are less conspicuous and perhaps already less numerous. Osteoclasts are clearly visible in the area of erosion. Although marked changes in size or arrangement of bony trabeculae have not yet occurred, the trabeculae are already somewhat coarser and less numerous, as they can not in every case be seen to be continuous with the cartilage matrix between adjacent cartilage cell columns.

The condition at the epiphysis of the hypophysectomized rat remains fairly constant between 6 and 10 days postoperative, and the status 8 days after operation has been chosen for illustration of this period (fig. 2). A slight further decrease³ in width of the epiphyseal cartilage has occurred, as will be seen in figure 2, and also by consulting table 1. The zone of undifferentiated hyaline cartilage is unchanged. The basophilic cells are smaller. The cell columns of the vesicular zone are still composed of three to four layers. These cells do not enlarge as much as in normal rats, and, as pointed out by Ingalls and Hayes, are less sharply polygonal and more rounded. The last layer of the vesicular zone is no longer highly vacuolated, and so does not form a distinct layer as it does in the normal rat or even in the rat 4 days after hypophysectomy. Cytoplasm and nuclei of cartilage cells are still visible in the cartilage cell spaces at the line of erosion. The number of capillaries directed against the cartilage has definitely decreased; they are no longer directed against each cell column. The bony trabeculae are broader, and most of them are now continuous with three or more columns of cartilage, instead of being continuous with the matrix between individual columns. The trabeculae are irregular in width, anastomose freely, and are quite close together, although marrow spaces

³ The decrease in width is not quite so marked as indicated by figure 2, as some shrinkage has occurred in the zone of flattened cells.

in this region are now much larger than at the time of operation. Osteoclastic activity can be noted near the erosion zone as well as on the surface of trabeculae. Bone formation in the erosion zone has ceased in some places, leaving the vesicular zone in direct contact with the cellular marrow. Osteoblasts have decreased in size and number and are less easily distinguishable than they were 4 days after operation.

TABLE 1

Width of the proximal epiphysis of the tibia in female rats.

NORMAL			HYPOPHYSECTOMIZED AT 26-28 DAYS OF AGE			
No. of animals	Age at autopsy	Cartilage width	No. of animals	Postoperative interval	Age at autopsy	Cartilage width
	<i>da.</i>	μ		<i>da.</i>	<i>da.</i>	μ
6	26	581				
6	30	502	6	4	30	398
			4	6	34	294
			4	8	36	302
			4	10	38	297
3	42	561	4	14	40	239
			6	18	46	195
1	56	345	1	28	56	231
6	65	248	5	36	62	209
2	74	267				
			1	56	84	224
1	119	195	1	91	119 ¹	198
2	123	220	8	100	126	215
1	160	172	1	132	160	178
2	170	145	4	145	168	208
1	195	163	1	167	195	186
1	251	132	24	205-210	230	211
15	305	120	1	272	300	173
			40	300-330	341-392	160
1	465	122	14	442	470	156
1	502	77				
6	546	99				
			2	617	650	153

¹ Measurements in older hypophysectomized rats include the calcified cartilage and true bone which seal the cartilage from the marrow.

Figure 3 shows the condition 14 days after operation. The condition is typical of the period from 14 to 18 days postoperative. It will be noted that the changes observed after 6 to 10 days are now more pronounced. The cartilage has continued to decrease in width. The basophilic flattened cells are separated by increased amounts of matrix. Their grouping is beginning to suggest the wedges or cones described

by Dawson ('29) in the old normal rat; this is accentuated by the greater amounts of matrix near the epiphyseal bone. Two to four layers of cells still show some enlargement but little vacuolation. There is a complete reversal in the proportion of bone and marrow in contact with cartilage, the marrow now occupying more of the cartilage interface than the bony trabeculae. Between these trabeculae, cartilage columns end abruptly at the marrow; capillaries are quite inactive in the erosion of such areas and little bone is being formed. Osteoblasts are flattened and appear inactive. The cartilage is still as broad as that of a 74-day-old normal rat, but cartilage morphology indicates far less activity. Capillary erosion is also less active than in the normal rat at 74 days, and the number of trabeculae is already greatly reduced, being more comparable to that in normal rats older than 1 year. The trabeculae in the hypophysectomized rat are, moreover, very delicate and usually contain less enclosed cartilage than in the normal rat; in fact, only delicate strands of cartilage matrix are present. The marrow shows increased numbers of fat cells. A few cartilage cells in the erosion zone are being encased by calcification of the intercartilaginous matrix; thus the initial phase of the "sealing off" process, by which the cartilage is ultimately completely separated from the marrow, has begun. Freud, Levie and Kroon observed in rats hypophysectomized at 45 days of age that a bony plate separated cartilage from marrow within 1 month. Ingalls and Hayes observed it within 13 to 30 days in rats hypophysectomized at 8 weeks of age.

Figure 4 shows the condition in the epiphyseal region 36 days after hypophysectomy. The cartilage has not decreased much in width but the cells are smaller and the amount of matrix has increased. The calcification of the marginal matrix has progressed so that it almost completely seals the cartilage from the marrow. Cellular marrow extends up to this calcified cartilage, but capillaries are no longer directed parallel with the columns of cartilage cells. The trabeculae of bone are sparse.

Figure 5 shows this region 100 days after operation. The width of the cartilage has remained fairly constant, but the decrease in cell size, especially in the zone of flattened cells, and the increase in matrix have progressed. The conical grouping of cartilage cells is now marked.

Figure 6 shows the condition 145 days after hypophysectomy. The cartilage is not apparently narrower than it was 14-18 days after operation. This specimen illustrates the variation in the "sealing off" process. Here, considerable stretches of uncalcified cartilage come directly into contact with cellular marrow. Even in such areas, capillaries

are not seen directed toward the cartilage columns, and may in fact be seen running parallel with the surface of the cartilage. The "sealing off" process is still chiefly the result of calcification of cartilage matrix rather than the result of osteoblastic activity. Bony trabeculae are sparse, and new formation has ceased for the main part, with only a few flattened osteoblasts still recognizable.

Figure 7 shows the condition 205 days after operation. The cartilage width is not measurably narrower, but the cells, even of the vesicular zone, are reduced in size and number. This is compensated by a further increase of matrix. Throughout the vesicular zone there is an increased basophilia of the matrix, possibly indicating partial calcification. Broad non-cellular areas of matrix are seen, sometimes extending through the entire width of the cartilage. Marrow still contacts cartilage in isolated areas. The fact that the calcified layer which "seals off" the epiphyseal cartilage has not become continuous and still varies from animal to animal in thickness, indicates some activity in remodeling. The constituents of the trabeculae also indicate the continuance of activity. Calcified cartilage matrix, which must have originated in some cartilage activity, is seen in limited amounts within the trabeculae. The trabeculae, as in the normal, are surrounded by true lamellar bone. Lamellar bone can also be seen on the surface of the calcified cartilage at the interface between cartilage and marrow.

Figures 8 to 14 illustrate the condition at the epiphyseal region, at approximately 50-day intervals, from a postoperative age of 272 to 617 days. Changes are relatively slight during this period, some of the differences illustrated being due to individual variability. The cartilage does not decrease further in width as is the case in the ageing normal rat, having reached the minimum width at the beginning of the period under discussion (272 days postoperative). The actual measurements of epiphyseal cartilage widths may be found in table 1. The cartilage is now sealed from the marrow by a much thicker layer of calcified material, and from this time it remains relatively constant in thickness.

Figure 8, illustrating the region 272 days after operation, shows that the calcification of the "sealing off" layer may involve all of the non-cellular matrix, or may be confined to the matrix immediately surrounding the cartilage cells. On the surface of the "sealing off" cartilage, as on the surfaces of the trabeculae, lamellar bone is found.

Figure 9 shows the region of the epiphysis 326 days after hypophysectomy. It will be noted that even after this period capillaries occasionally extend through the layer of "sealing off bone". An unusually large mass of cartilage matrix — apparently uncalcified, as far as can

be told from the hematoxylin and eosin stain — is seen extending into one of the bony trabeculae. Usually the amount of cartilage enclosed in the core of the trabeculae is much more restricted than in this instance which is more nearly comparable with the condition seen in normal rats. Another thing of interest in this section is the increased basophilia (ordinarily associated with calcification) in the matrix of the whole vesicular zone. This sharply demarcates the vesicular zone from the zone of flattened cells.

Figure 10 shows the epiphyseal disc 376 days after operation. The calcification of the matrix throughout the vesicular zone is also shown here. An occasional capillary can be seen penetrating through the "sealing off" bone and even through the calcified vesicular zone. When seen in cross section these capillaries appear as islands in the attachment of the trabeculae or in the "sealing off" bone.

Figure 11, illustrating the proximal epiphyseal region of the tibia of a rat 442 days after hypophysectomy, shows very clearly the wide stretches of cartilage between trabeculae. The line of demarcation between cartilage and marrow, in the intervals between trabeculae, is regular and clearly defined, and is characteristic of the old hypophysectomized rat. The sharpness of the demarcation is in marked contrast to the irregularity of the cartilage margin in old normal rats.

Figure 12 shows the epiphyseal plate 506 days after operation. Here, too, the matrix of the whole vesicular zone has been calcified. A higher power view of this region in another animal of the same postoperative period is shown in figure 15. It shows well the calcification of the matrix immediately surrounding the cartilage cells.

Figure 13 shows the epiphyseal plate after a lapse of 561 days from the time of operation. The thin layer of lamellar bone on the surface of the calcified (metaplastic?) cartilage is clearly illustrated here. The trabeculae, on the other hand, consist, as far as can be seen, entirely of lamellar bone.

Figure 14 shows the area after the longest interval studied, 617 days. The columnar arrangement of the cartilage is almost lost, due to a number of factors: the few cells present, the large amounts of matrix, and the uneven distribution of matrix in relation to cells. The "sealing off" bone in this case is thinner than in any other example from these longer postoperative periods. Further, the matrix of the vesicular zone does not appear highly calcified. The continuance of cartilage into some of the trabeculae, together with some evidence of osteoblastic activity on the surface of the trabeculae, indicates that some growth and transformation is continuing, however slowly, even after this long post-

operative period. The increased numbers of fat cells characteristic of the marrow of hypophysectomized rats is well illustrated here.

SUMMARY

Female rats were hypophysectomized at a constant age, 26 to 28 days, and were sacrificed at intervals to determine the progressive changes in endochondral bone formation, as typified at the proximal epiphysis of the tibia. The conditions observed in the cartilage plate and the trabecular bone, primary and secondary spongiosa, have been described and illustrated at postoperative ages ranging from 4 to 617 days.

The first change observed is a thinning of the cartilage plate, due to decrease in number but particularly in size of cells. This is seen within 4 days after hypophysectomy, and the change is progressive until 250 to 300 days after operation, after which very little further decrease in cartilage width occurs. Concomitant with decrease in cell size there is a progressive increase in amount of matrix. Further, groups of cell columns become separated by masses of matrix, and as the increase in matrix is more voluminous nearer the epiphyseal lamina of bone, and as the cells even in the zone of flattened cells are slight enlarged toward the diaphyseal side, the groups of cartilage columns assume a conical shape, the bases of the cones being toward the diaphysis. The cartilage forms a continuous epiphyseal plate across the entire bone for the maximum period studied, 617 days after operation.

A decrease in vascularity at the line of erosion is observed by the fourth day postoperative; however, degenerate cartilage cells are still seen terminating each cartilage column, which indicates that active erosion is still occurring. The trabeculae are, however, already slightly coarser even at this brief postoperative period, and alignment of trabeculae with each intercolumnar spicule of calcified cartilage matrix can no longer be traced. Eight days after operation the trabeculae are definitely coarser and farther apart. By 14 days after operation, resorption of trabeculae has progressed so far that there are wide marrow spaces between them. The trabeculae after longer postoperative intervals become very sparse, more so than in very old normal rats. As the trabeculae become coarser and less numerous they are seen to be continuous with two or three cartilage columns; cartilage matrix, including some cells, extends down into the cores of the trabeculae. This phenomenon is, however, never so conspicuous and constant as in old normal rats.

The boundary between cartilage and marrow in the wide spaces between trabeculae is sharply defined. At this interface cartilage columns end abruptly; no response of cells (enlargement or vacuolation) occurs, and no erosion of the cartilage cells by the capillaries of the marrow can be seen. The abruptness of this interface is further accentuated by the calcification of the marginal cartilage. The process of "sealing off" the cartilage from the marrow initially involves only the matrix between the terminal cells of the columns; later the matrix throughout the vesicular zone becomes calcified (as far as can be judged from the depth of stain with hematoxylin). Occasionally the calcification involves only the capsule or matrix immediately around the cells of the vesicular zone, and the cartilage cells appear as calcified globules embedded in normal matrix. Some cells of the vesicular zone always remain unchanged by this process, at least up to this time. The beginning of this "sealing off" process is seen as early as 14 days post-operatively. There is a good deal of variability in the time of appearance and in the thickness of this layer of calcified cartilage; the layer is sometimes intermittent indicating some activity and remodelling as late as 250 to 300 days after operation. After this period, when the matrix of the vesicular zone has become completely calcified, the "sealing off" layer remains more consistently a broad transverse band along the margin of the cartilage. Even 300 days after hypophysectomy, capillaries may be seen penetrating the whole width of the vesicular zone. The lamina of calcified cartilage which seals cartilage from marrow should perhaps be considered as bone formed by metaplasia of cartilage. Some true lamellar bone is superimposed upon it. The trabeculae consist almost entirely of lamellar bone.

The conditions seen at the proximal epiphysis of the tibia at progressive intervals following hypophysectomy resemble strongly those seen in ageing normal rats. There are however, some marked differences. The hypophysectomized rat differs greatly in the speed with which the changes overtake the epiphyseal region. The osteoblasts, for example, atrophy and almost completely disappear very soon after hypophysectomy, whereas in normal ageing, this is a very gradual process. Furthermore, the cartilage plate decreases in width at first very abruptly, then quite slowly to about 272 days from which time it remains at a width greater than that of the normal rat of the same age. Likewise, soon after operation, the trabeculae become sparser than in the very old normal rat. Only small amounts of cartilage are enclosed within the bony trabeculae, whereas ample inclusions are characteristic of the older normal rat. In the hypophysectomized rat the boundary line

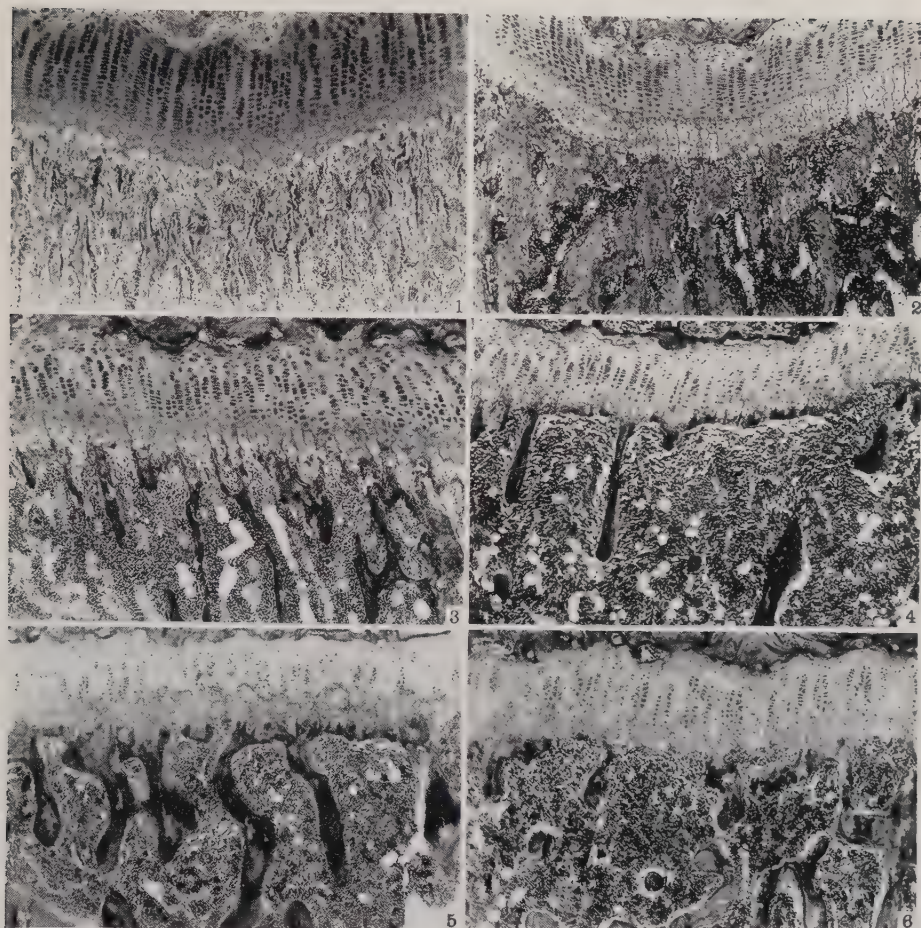
between cartilage and marrow is very sharp, whereas in the normal rat, the continuity of the cartilage into the core of the more numerous trabeculae produces a markedly irregular outline. Some "sealing off" of cartilage from marrow occurs in the normal rat but is never as extensive or consistent, as in the hypophysectomized rat. The calcification of the whole vesicular zone, common in the hypophysectomized rat, is not seen in the normal. The enclosure of cartilage cells in globular masses of calcified matrix, characteristically seen in the normal rat after 250 days of age, is not a consistent phenomenon in the hypophysectomized rat.

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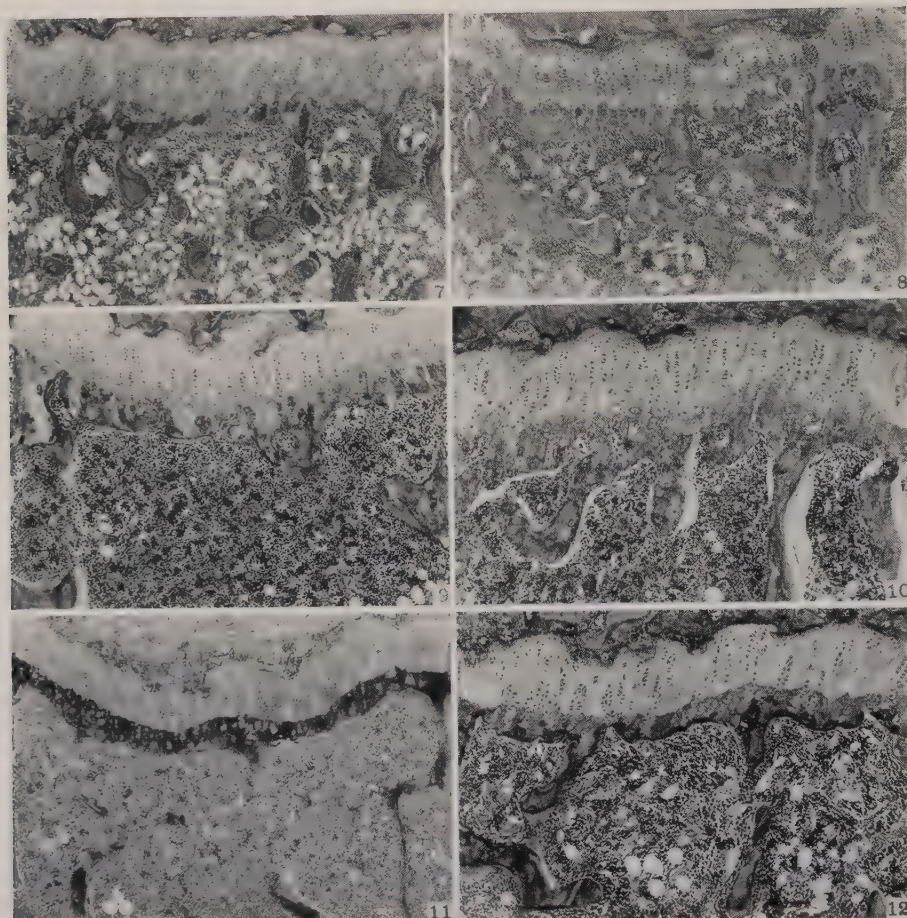
EXPLANATION OF PLATES

Proximal epiphyseal region of the tibia of female rats hypophysectomized at 26 to 28 days of age and kept for various postoperative intervals. H. and E. stain, $\times 56$.



EXPLANATION OF FIGURES

1	Postoperative interval	4 days.	Sp. 6752A.	Pl. 8480
2	Postoperative interval	8 days.	Sp. 8689.	Pl. 8481
3	Postoperative interval	14 days.	Sp. 6515.	Pl. 8482
4	Postoperative interval	36 days.	Sp. 7366.	Pl. 8491
5	Postoperative interval	100 days.	Sp. 7663.	Pl. 8483
6	Postoperative interval	145 days.	Sp. 7807.	Pl. 8484



EXPLANATION OF FIGURES

7	Postoperative interval 205 days.	Sp. 8521.	Pl. 8486
8	Postoperative interval 272 days.	Sp. 8382.	Pl. 8345
9	Postoperative interval 326 days.	Sp. 6879.	Pl. 8492
10	Postoperative interval 376 days.	Sp. 6882.	Pl. 8496
11	Postoperative interval 442 days.	Sp. 7881.	Pl. 8169
12	Postoperative interval 506 days.	Sp. 6882AA.	Pl. 8488



EXPLANATION OF FIGURES

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|----|--|-----------|----------|
| 13 | Postoperative interval 561 days. | Sp. 7874. | Pl. 8489 |
| 14 | Postoperative interval 617 days. | Sp. 6575. | Pl. 8490 |
| 15 | Higher magnification ($\times 281$) of the epiphysis of a rat of the same postoperative age as shown in figure 12. Sp. 6844. Pl. 8112. | | |

A STUDY OF THE GENESIS OF HISTOLOGICAL CHANGES PRODUCED BY CALORIC RESTRICTION IN PORTIONS OF THE ENDOCRINE AND REPRODUCTIVE SYSTEMS OF STRAIN "A" FEMALE MICE¹

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THREE TEXT FIGURES AND THREE PLATES (NINE FIGURES)

INTRODUCTION

A previous paper (Huseby, Ball and Visscher, '45) reported the results of a histological study of portions of the reproductive and endocrine systems of 17- to 18-month-old C3H mice maintained from the time of weaning on a diet which restricted caloric intake to two thirds that of the ad libitum fed control animals. Although the production of pituitary insufficiency by inanition has been well established (Marrian and Parkes, '29; Mason and Wolfe, '30; Moore and Samuels, '31; Pomerantz and Mulinos, '39; Werner, '39; Mulinos and Pomerantz, '40, '41), and the over-all histological picture presented by our previous material was essentially that described in rats (Mulinos and Pomerantz, '40), a few points of difference were noted. The ovaries of the restricted animals contained an abundance of small and medium sized follicles while those of the control group were almost devoid of these elements. No corpora lutea were found in the restricted animals. No wheel cells or yellowish-green pigment was observed in the adrenals of the under-fed group.

Because of these differences and because, to our knowledge, there has been no previous histological study of the reproductive and endocrine systems in chronic inanition instituted before sexual maturity, the present investigation was undertaken.

MATERIALS AND METHODS

Immature female mice of the A strain maintained in this laboratory were placed on the experiment when 23 to 29 days of age. All animals

¹ These studies were supported by the Sivertsen Foundation and the Graduate School Fund for Cancer Research.

were individually caged in galvanized iron cages, partly wire mesh and partly sheet. Water was available at all times. The caloric intake of half of the animals was controlled by feeding weighed amounts of the "restricted diet" (table 1), each animal receiving 1.36 gm. per day for the first 2 weeks and 1.51 gm. per day thereafter. The other animals were allowed to consume the "control diet" ad libitum. Previous observations (Visscher, et al., '42) have shown that on this regime the restricted animals obtain approximately the same amount of protein, vitamins, and salts as the ad libitum fed control animals but are calorically restricted by about one third.

TABLE 1¹

COMPONENT	CONTROL DIET GM./100	RESTRICTED DIET GM./100
Glucose	33	22.0
Lard	20	15.3
Casein	28	37.4
Dry yeast (Anheuser-Busch Strain G7)	8	10.6
Dry alfalfa leaf	4	5.4
Salt mixture ²	7	9.3

¹ Each animal was given a supplement of 0.1 cc. of U.S.P. XI cod liver oil and the same amount of wheat germ oil each week added directly to the food cup.

² CaCO₃—543, MgCO₃—25, MgSO₄—16, NaCl—69, KCl—112, KH₂PO₄—212, FePO₄—21, KI—0.08.

All animals were weighed every 3 days, and groups of five restricted² and five ad libitum fed animals were sacrificed after 1, 2, 3, and 4 weeks and 2 and 4 months. The ovaries, uteri, and adrenals were fixed in Bouin's solution, dehydrated in alcohol, imbedded in paraffin, sectioned at 5 micra, and stained with Delafield's hematoxylin and eosin. Multiple sections were made through the ovaries and adrenal glands while sections of the uterine horns were taken from the middle third only in order to obtain comparable areas in all animals. The mammary histology was studied by means of both section and whole mount techniques.

RESULTS

Body weight

At the beginning of the experiment the average body weight of the mice in the restricted and ad libitum fed control groups was 6.7 gm. During the first week the fully fed animals gained weight very rapidly

² Due to the death of two of the restricted animals before the designated time of autopsy only four animals are included in the 2 and 4 months restricted groups.

to average 12.3 gm. at the end of the week, but thereafter weight gain was slower so that after 2 months the ten living animals averaged 21.1 gm. The restricted animals, on the other hand, gained weight slowly throughout the experiment to average 13.8 gm. at the end of 2 months (8 animals). The composite weight curve for these animals, given in figure 1, corresponds closely to that previously reported for C3H mice (Vissscher, et al., '42) in spite of a rather large difference in initial weights in the two experiments: 6.7 gm. vs. 11.3 gm.

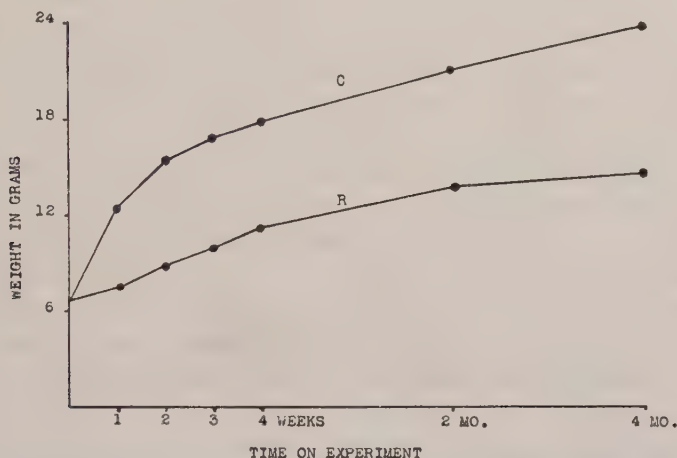


Fig. 1 Mean body weights in relation to age of A strain virgin female mice. Curve C presents the values for the control diet; curve R for the restricted diet.

Ovaries

To avoid confusion due to differences in nomenclature, it seems advisable to define some of the terms to be used in this description. The term "interstitial cell" will be used to refer to those cells of the stroma that have an abundant, vacuolated, eosinophilic cytoplasm and are morphologically very similar to the cells of the adult corpora lutea. These cells are very abundant in mouse ovaries, particularly mice of the A strain, forming rather large clusters throughout the stroma of the adult organ. The other cells of the stroma will be referred to as "stromal cells" or "stromal connective tissue cells". The term "luteinizing follicle" will be reserved for those follicles whose granulosa cells are differentiating to luteal cells while the ovum is degenerating. Such follicles can be differentiated from true corpora lutea developing after follicular rupture only by the presence of degenerating ova, although their position nearer the center of the ovary is often also suggestive.

The ovaries of seven mice 23 to 25 days of age were studied to ascertain the ovarian histology at the beginning of the experiment. At this age the ovaries are crowded with follicles in all stages of development from primary follicles to those containing a moderately large antrum. The cortex is rather distinct from the medulla, and the cortical stroma is confined to narrow bands between the follicles. At this stage the stroma is composed entirely of stromal connective tissue cells and vessels. Some follicular degeneration is present. This occurs mostly through the invasion of the follicle by a cluster of large connective tissue cells with leptochromatic nuclei. Rather uncommonly, however, following degeneration of the ovum, the cells of the granulosa differentiate to cells having a characteristic spoke-like nuclear pattern (wheel cells of Selye, Collip and Thomson, '33).

Ovaries of the ad libitum fed control mice. After 1 week the ovaries of the fully fed mice have changed but little; the amount of stroma in the interfollicular spaces and follicular degeneration resulting in wheel cell formation have increased slightly. At the end of 2 weeks a definite increase in follicular degeneration has occurred, and the stroma has continued to increase becoming slightly looser in appearance. An ovary of one mouse of this latter group 42 days of age contained a luteinizing follicle.

In the group studied after 3 weeks, the connective tissue stroma has not only increased markedly in amount but also has become much looser in appearance and contains clusters of cells which have vacuoles throughout their cytoplasm. There is a marked increase in the number of partially degenerated follicles especially those of smaller size whose granulosa cells transform to wheel cells. The wheel cells thus formed tend to remain in small clumps and transitional forms between them and stromal connective tissue cells can be found about these clusters, figure 6. Several corpora lutea and/or luteinizing follicles are present in ovaries of this group, and the number of follicles per ovary has decreased, presumably due to degeneration. In one ovary two luteinizing follicles were found in which the centers were composed of a degenerated ovum surrounded by wheel cells, figure 4. Transitional forms between the wheel cells in the centers and the normal young luteal cells at the periphery were present.

One month after the beginning of the experiment, when the mice were 7 to 8 weeks of age, the ovaries of four of the five ad libitum fed animals studied contain well developed corpora lutea, figure 5. In the stroma the clusters of cells that a week earlier exhibited vacuolation of the cytoplasm have increased in size, contain more vacuoles and are

slightly eosinophilic, appearing morphologically similar to young luteal cells and are, therefore, to be considered as young interstitial cells. Follicular degeneration with wheel cell production is of approximately the same degree as at the 3-week period. The one animal that had no corpora lutea, although it has a satisfactory initial weight gain, had lost 1 gm. in weight during the 10 days immediately preceding autopsy. In addition to the absence of corpora lutea, the stroma of its ovaries was more compact containing no young interstitial cells and only relatively few cells with the vacuolated cytoplasm noted in the 3-week group.

The ovaries of the 2- and 4-month groups will be discussed together as they have essentially the same histology. They are considerably larger than those of the previous groups, each ovary contains several young and mature corpora lutea, and their stromata are packed with clusters of mature interstitial cells. Follicular degeneration is not common, and there is only an occasional small nest of wheel cells. The number of normal follicles in all stages of development has not changed notably from that in the 1-month group. The ovaries in one animal in the 2-month group, however, presented a rather different picture from that described above. This animal had been losing weight for 5 days previous to autopsy at which time it was the lightest animal of the group and weighed the same as it had 18 days before. Its ovaries, as in the mouse of the 1-month group that had been losing weight, contained no corpora lutea. The stroma was compact with no mature, or even young, interstitial cells; and there were more nests of wheel cells present than in the ovaries of the other animals in the group.

Ovaries of the restricted animals. The most noticeable change occurring in the ovaries during the first week of caloric restriction is in the stroma, which has become more compact. The stromal connective tissue cells have decreased in size with some of the nuclei becoming more pachychromatic and the chromatin tending to assume a spoke-like pattern suggestive of transition to wheel cells. There is very little change in the follicles. Follicular degeneration appears to be of the same magnitude as in the ovaries of the fully fed mice at this age. In the large follicles there are some granulosa cells degenerating through nuclear pyknosis, but these are seen no more frequently than during antrum formation in normal follicles.

During the 2- and 3-week periods the ovaries of these mice do not change markedly. The stroma becomes slightly more dense with some of the stromal cells assuming a typical wheel cell appearance. There is an increase in the rate of follicular degeneration producing wheel cells roughly paralleling that seen in the fully fed animals of corresponding

ages. This process in restricted animals is slightly different however, in that somewhat larger follicles undergo this type of change, and the wheel cells when formed do not remain localized in small nests but are disseminated by connective tissue while still retaining their characteristic morphology. There appear to be, therefore, two separate sources for the wheel cells scattered throughout the stroma, namely, from the granulosa cells of degenerating follicles and from the stromal connective tissue cells.

In the ovaries of the mice autopsied after 1 month of dietary restriction (figs. 7 and 8) the stroma, although still as compact as in the previous groups, is more abundant and comprises a relatively large proportion of the glands. The production of wheel cells through follicular degeneration has reached its peak at this time, exceeding that seen in any of the *ad libitum* fed groups or in the restricted animals studied at other periods. Although the number of larger follicles has decreased, younger follicles are more numerous than in the ovaries of the *ad libitum* fed animals at this age so that the total number of follicles per ovary is approximately the same in the two groups. However, as no corpora lutea or interstitial cells are present in the ovaries of the restricted mice, the difference between them and the ovaries of the *ad libitum* fed group of the same age, in which both elements are prevalent, is more striking than at any previous period.

At the end of 2 months of dietary restriction, follicular degeneration has decreased noticeably but is still in excess of that seen in the corresponding *ad libitum* fed group. Young follicles are very abundant. In contrast to the ovaries of the fully fed animals no corpora lutea are present. The stroma, although increased in amount, is still as compact as previously and contains no interstitial cells.

The greatest change in the ovaries of the 4-month group is in the stroma which has increased considerably in amount and now has a looser appearance. Although there are still numerous wheel cells dispersed throughout, many of the stromal cells have increased in size particularly through an increase in cytoplasm, which often contains small vacuoles. There are, however, no cells present that could be designated as young interstitial cells. The status of the follicles is much the same as in the preceding group, there being a predominance of younger follicles, some follicular degeneration with wheel cell production, and again no corpora lutea. The total number of follicles of all types present in the two groups of animals at this age is approximately the same.

Summary of ovarian differences. The most striking feature of the ovaries of the restricted group is the absence of both corpora lutea and interstitial cells in the thirteen mice studied after 50 days of age during which time these elements are prevalent in the ovaries of the fully fed mice. Although from the first week of the experiment the ovaries of the restricted animals are somewhat smaller than those of their ad libitum fed controls, because corpora lutea and interstitial cells constitute such a great part of normal ovaries, the size difference in the two groups is very marked after the control mice reach sexual maturity. Thus in the 1-, 2-, and 4-month groups the ovaries of the restricted mice present a cross section only half to two thirds as large as their fully fed controls.

The process of follicular degeneration in the two groups is also rather different. Although small follicles in the control ovaries often degenerate with the production of wheel cells, the nests of wheel cells are never large and the wheel cells themselves appear to differentiate into normal stromal cells. The degeneration of larger follicles is either by luteinization or by connective tissue invasion, i.e. invasion of the follicle by a clump of large connective tissue cells. In the restricted animals, on the other hand, somewhat larger follicles, as well as the smaller ones, produce wheel cells during degeneration, and the nests of wheel cells so formed appear to become broken up by the general connective tissue stroma while the wheel cells retain their characteristic morphology. The degeneration of the larger follicles, those corresponding in size to medium sized follicles in normal adult ovaries, is always through invasion by large connective tissue cells, luteinization not having been observed.

In spite of the decreased number of larger follicles in the restricted animals, because of the presence of a greater number of smaller ones, the total number of follicular elements in the two groups is approximately the same throughout the experiment.

Uteri

The uteri of the mice 23 to 25 days of age are small in diameter with an undeveloped endometrium. The luminal epithelium is low columnar with the nuclei, which are toward the basement membrane, forming a regular palisade. The endometrial glands are few in number and of small diameter with no signs of proliferative activity. The lamina propria is rather loose in appearance, but not edematous, and contains few if any mitotic figures. The myometrium is compact and composed

of small muscle cells with pointed nuclei surrounded by scanty cytoplasm.

Uteri of the control animals. For the first 2 weeks of the experiment the uteri of the ad libitum fed animals increase gradually in size with no striking deviation from the "starting point" picture. The height of the endometrial epithelium increases somewhat, mitoses can be seen occasionally, and the nuclei are not as regularly arranged toward the basement membrane as they were previously. The tunica propria is virtually unchanged except for some increase in amount, but the cells of the myometrium have hypertrophied noticeably, and the nuclei have increased in size and have become more blunt.

After the third week, when the mice are 6 to 7 weeks of age, the uteri undergo changes indicating the approach of sexual maturity. They increase rapidly in size. The endometrial epithelium, both luminal and glandular, becomes tall columnar with frequent mitotic figures and many of its nuclei migrate toward the lumina indicating the beginning of cyclic changes. The glands increase in size and number, and the lamina propria becomes looser in appearance showing signs of proliferation. The myometrical cells continue to hypertrophy.

By 1 month the uteri are almost mature histologically, figure 9. There are variations from animal to animal as to the height of the epithelium, the position of its nuclei and the looseness of the lamina propria indicating that cycles have been established. In the 2- and 4-month groups sexual maturity has been attained, for in various uteri of the ten animals studied all stages of the estrous cycle can be identified.

Uteri of the restricted animals. During the first week of dietary restriction the uteri decrease slightly in size, and the lamina propria becomes dense with some of the cell nuclei presenting an atypical wheel cell pattern. The growth during the subsequent periods is only slight, figure 10, for after 2 months when the uteri of the fully fed animals have increased greatly in size and have begun to show cyclic changes, those of the restricted group are essentially the same size as in the animals 23 to 25 days of age with no increase in glandular development and a lamina propria that is very compact and contains some rather typical wheel cells. The uteri of all restricted animals show a picture similar to that seen in castration, with no glandular development, a low columnar glandular and luminal epithelium without signs of proliferation, and a dense lamina propria. The myometrium remains undeveloped being composed of closely packed small muscle cells with rather pointed nuclei.

Mammary glands

During the first month mammary development in the fully fed mice consists mostly of extension and branching of the larger ducts by means of end bud proliferation. This growth is rather slight for the first 2 weeks of the experiment but increases as the animals approach sexual maturity. By 2 months, when the animals are 11 to 12 weeks of age, the glandular tree has increased greatly in complexity, and a few small lateral buds can be seen along the larger ducts, figure 2. The glands

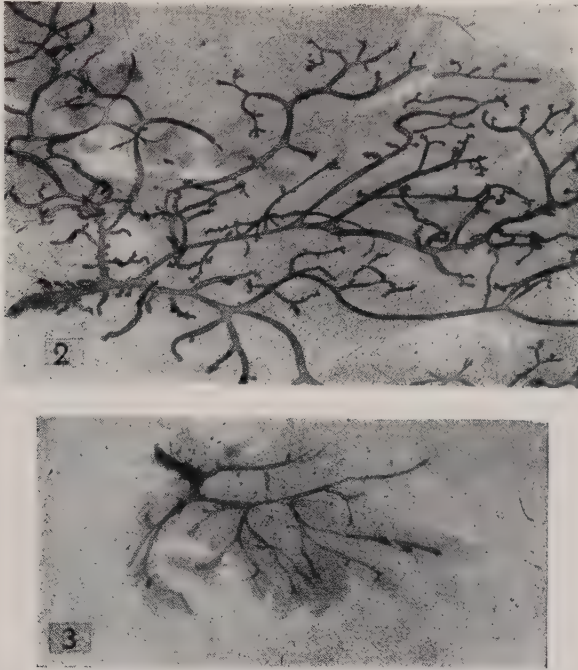


Fig. 2 A portion of a mammary whole mount of a control animal of the 2-month group. The pattern of the mammary tree is rather complex and there are a few lateral buds present on the larger ducts. $\times 10$.

Fig. 3 A rudimentary mammary gland of a restricted animal after 2 months on the experiment. $\times 10$.

of the 4-month group are fully developed for virgin mice of this strain. The ducts extend throughout the mammary fat pads, there are many side branches of small caliber, and a goodly number of lateral buds are present in all areas.

In the restricted group, on the other hand, the glands remain rudimentary during the first month with but little evidence of duct extension. At 2 months there is some variation from animal to animal. In

some, glandular growth has begun to a slight degree, the area of the glands having increased to two or three times that of the rudimentary glands. In all such glands, however, the ducts are thin with but very few side branches and no lateral buds. In other animals the mammae have grown but little and are still rudimentary in character with no end buds to indicate active growth, figure 3. By 4 months the mammae of the restricted animals have developed considerably from those seen at 2 months but are still very much less developed than in the 4-month ad libitum fed animals. Their duct system has extended considerably, but there are still relatively few side branches and almost no lateral buds giving the glandular tree as a whole a rather simple pattern and the individual ducts a very smooth contour. An occasional gland may be found that is still essentially rudimentary but exhibits numerous large end buds indicating the beginning of more extensive duct growth. Thus the growth of the mammary rudiments in the restricted mice is apparently greatly retarded in time of onset and once initiated produces generally underdeveloped glands with few side branchings and almost no lateral buds.

Adrenal cortices

In the cortex proper there is little difference in the two groups. The cortices of the restricted animals are somewhat thinner than their corresponding controls, and the cytoplasm of the cortical cells is slightly less plentiful with a smaller amount of lipid vacuolization. In none of the restricted animals was the yellowish-green pigmentation or wheel cells described in rats (Mulinos and Pomerantz, '40) observed.

Striking differences, however, are to be noted in the juxtamedullary or X-zone in the two groups. This zone is present in all of the animals studied at 23 to 25 days of age but has as yet not reached its full development. In the ad libitum fed animals it increases somewhat in thickness during the first week or so and is retained at this stage for 3 weeks when a few large, clear fat cells appear in it indicating the beginning of degeneration by vacuolization, figure 11. This vacuolization increases gradually through the subsequent periods until at 4 months, when the animals are approximately 150 days old, about 80% of the X-zone has been replaced by fatty tissue.

After 1 week of diet restriction, however, only two of the five mice studied had recognizable X-zones. In one of these, the zone was about the size seen at the beginning of the experiment, while in the other it was considerably smaller. By 2 weeks the X-zone had completely disappeared in four of the animals and was greatly reduced in the fifth,

and in all adrenals studied during the remainder of the experiment no X-zone was seen, figure 12. The degeneration of this zone differs from that seen in the ad libitum fed animals in that vacuolization does not occur. Instead the small eosinophilic cells which constitute the juxta-medullary zone are replaced by connective tissue without showing histological signs of cellular degeneration.

DISCUSSION

In following the changes in the ovaries of mice placed on a calorically restricted diet before they reach sexual maturity, it is of interest to note that at no time is there excessive follicular degeneration. Although the larger follicles gradually decrease in number, there is a compensatory increase in primary and secondary follicles so that at any given time these ovaries contain approximately as many follicular elements as do those of normally nourished animals. It would thus appear, as has been suggested by several authors (Loeb, '17; Papanicolaou and Stockard, '20; and Mulinos and Pomerantz, '40), that it is the older follicles that are primarily affected by the decreased pituitary gonadotropin production brought about by inanition. Contrary to the findings of Russo ('06) in rabbits, the germinal epithelium showed no signs of degeneration, and evidence of ovum production by this epithelium could be found in all restricted animals studied. The primary follicles appeared entirely normal and developed normally, as far as could be determined, until they possessed several layers of granulosa cells surrounding the ovum. Degeneration of follicles larger than this, however, is the common thing with only few follicles reaching the stage of moderate antrum formation.

It would appear that inanition, instead of causing an ovarian degeneration, actually preserves the ovary in a pseudo-juvenile condition. This concept is supported histologically by the picture seen in mice 17 to 18 months of age (Huseby, Ball and Visscher, '45) where the ovaries of those animals maintained on a restricted diet throughout the normal period of fertility possessed numerous normal appearing small follicles while the ovaries of their ad libitum fed controls were almost completely devoid of such elements. Thus it would seem that while the ovaries of normal mice gradually become depleted of follicles as they advance in age and become infertile, the ovaries of calorie-restricted animals are maintained for a long period of time in a condition somewhat suggestive of pre-puberty.

This concept was born out functionally when two groups of virgin mice, one of which had been maintained from weaning on a one-third

calorie-restricted diet, the other having been fed *ad libitum*, were bred at 10 months of age after the restricted animals had been placed on a calorically adequate diet (Ball and Visscher, unpublished data). Under these conditions the animals that had been restricted for 9 months had a much higher percentage of pregnancies than did their controls, which had been fed *ad libitum* throughout their entire life span.

The absence of corpora lutea in the ovaries of the thirteen calorie-restricted mice studied after the age of 2 months is not surprising since Smith ('30) found that whereas hypophysectomized adult animals retained corpora lutea for a long time, if this operation was carried out on sexually immature animals no corpora lutea developed. A question does arise, however, since when mice were maintained on a diet identical with that used in this experiment and continuously mated, 39% of the mice became pregnant once and 3% twice (Ball and Visscher, unpublished data). It is to be noted, nevertheless, that normal mice of this strain when "forced bred" usually average eight to nine pregnancies,³ so that these findings represent at least a 90% reduction in fertility. Thus our failure to find corpora lutea in the restricted mice of this experiment probably indicates that corpora lutea occur but rarely in animals as severely undernourished as these, and that we did not happen to autopsy an animal at the time when one of the infrequently occurring corpora lutea was present.

Since in this experiment the ovaries were studied as the mice passed through puberty, excellent material for the study of the origin of interstitial cells and their abnormal counterpart, wheel cells, was obtained. That the characteristic wheel cell of hypophyseal insufficiency is an abnormal counterpart of the normal interstitial cell has been shown by Selye, Collip and Thomson ('33) for when they injected gonadotropic substances into hypophysectomized rats the wheel cells differentiated into interstitial cells in those animals that did not possess corpora lutea. Further evidence for this is found in the present experiment in the occurrence of luteinizing follicles whose centers are composed of a degenerated ovum surrounded by wheel cells, figure 4. Here transitional forms can be seen between the wheel cells in the center and normal appearing young luteal cells at the periphery.

From observations in the literature dealing with interstitial cells one must conclude that not only the number of interstitial cells present but also the origin of these cells varies in different species (for literature

³ Such breeding records occur only when mammary cancer does not produce death at early ages. Normal A strain breeding females do not develop mammary cancer in the absence of the milk influence (Bittner, '36).

see Corner, '32). In the mouse they have been found to be derived from the granulosa directly in instances of follicular degeneration (Velloso de Pinho, '25) and indirectly through the breaking up of adult corpora lutea by connective tissue invasion (Velloso de Pinho, '25; Pfeiffer and Hooker, '42). The studies of the normal ovaries of mice of the 3-week and 1-month ad libitum fed groups in this experiment indicate that interstitial cells in mice may also be derived from the connective tissue of the general stroma. Thus in the 3-week control group, mice 44 to 50 days of age, in ovaries that had no luteinizing follicles or corpora lutea, clusters of stromal cells were present that contained a large amount of vacuolated cytoplasm. A week later when corpora lutea were present but were young and gave no signs of being broken up by connective tissue, clusters of cells morphologically identical with young interstitial cells were abundant throughout the stroma, and transitions were present between these young interstitial cells and vacuolated stromal cells as seen in the ovaries of the 3-week group. There is also similar evidence in the restricted animals that wheel cells are formed from the stromal connective tissue cells as well as from the granulosa cells of degenerating follicles.

The occurrence of small nests of wheel cells in the ovaries of apparently normal mice is also of great interest. To our knowledge all previous investigations in which they are described deal with conditions relative to hypophyseal insufficiency (Selye, Collip and Thomson, '33; Mulinos and Pomerantz, '40; Laqueur and Ludwig, '41), and the cell type is generally considered to be pathognomonic of a decreased pituitary secretion of gonadotropin. According to this reasoning it would be concluded that these normal animals at the time of puberty produced insufficient gonadotropic hormone to luteinize all the granulosa of the degenerating follicles.

The effect of inanition upon mammary development is first a retardation of the onset of development and second a definitive underdevelopment of the glands. After 2 months of dietary restriction, when the animals are 85 days of age, many of the mice still have glands that are rudimentary or just beginning to develop while the mammae of the control animals at this age are approaching the mature state. The main factors contributing to the underdevelopment of the definitive glands are, as previously discussed in detail (Huseby, Ball and Vischer, '45), two in number: the decreased production of ovarian hormones as indicated by the "castrate" adnexa, and the decreased response of mammary glands to estrogen in the presence of hypophyseal insufficiency.

The rapid disappearance of the adrenal cortical X-zone after the institution of caloric restriction is in accord with the observation by Deanesly ('38) of a complete absence of this zone in a strain of pituitary dwarf mice. Both of these observations suggest that the maintenance of the juxtamedullary zone is dependent upon hypophyseal function. It is also interesting that this zone in the restricted mice degenerates without vacuolization in contrast to the characteristic vacuolization of the zone in normal female mice of this strain. The length of time that this juxtamedullary zone persists in normal virgin females is also of interest since Daughaday ('41) observed it to have disappeared completely and without vacuolization by 100 days of age in mice of the low mammary cancer C57 Black strain while in the DbA strain, which has a high incidence of mammary cancer in both virgin and breeding females, it degenerated by vacuolization and was found to persist in some animals to 210 days of age. The A strain has a low incidence of mammary cancer when the females are kept as virgins but a high incidence when they are bred. The X-zone degenerates by vacuolization as in the DbA but at a time intermediate between that of the C57 Black and DbA strains, being approximately 80% replaced by 150 days. Thus a correlation may exist between the maintenance of the X-zone and its time of normal disappearance with vacuolization and a factor or factors important in carcinogenesis.

SUMMARY

1. The ovaries of mice maintained from the time of weaning on a diet that restricted caloric intake by one third, present a pseudo-juvenile picture in that they contain many follicles, particularly young follicles, but no corpora lutea or interstitial cells.
2. The uteri of these restricted animals fail to develop beyond the pre-pubertal state and present an appearance similar to those of castrates.
3. Mammary gland development is greatly retarded in onset, and the definitive glands are markedly underdeveloped having few small side branches and almost no lateral buds.
4. The juxtamedullary zone of the adrenal cortex rapidly degenerates without vacuolization after the institution of caloric restriction.
5. Evidence is presented indicating the origin of interstitial cells in mice from the stromal connective tissue cells as well as from the luteal cells of the old corpora lutea.
6. The appearance of wheel cells in the ovaries of normal young mice suggests a low level of pituitary gonadotropin at the time of puberty.

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PLATE 1

EXPLANATION OF FIGURES

4 An area of a control ovary from the 3-week group showing the luteinization of a follicle. The center is filled with debris, probably from a degenerated ovum, surrounded by wheel cells with transitional forms between these wheel cells and the young luteal cells of the periphery. $\times 310$.

5 A control ovary after 1 month on the experiment. This animal has reached sexual maturity the ovary containing several corpora lutea and many normal follicles. Note the areas of darkly staining cells. These are areas of wheel cells formed by follicular degeneration. $\times 37.5$.

6 A field from the ovary pictured in figure 5 showing a normal follicle and a normal corpus luteum. The group of small dark cells in the center is a degenerating follicle forming wheel cells. $\times 310$.

7 An ovary from the 1-month restricted group. Note particularly the dense stroma, the numerous normal follicles and the dark appearing areas indicating wheel cells formed by follicular degeneration. $\times 75$.

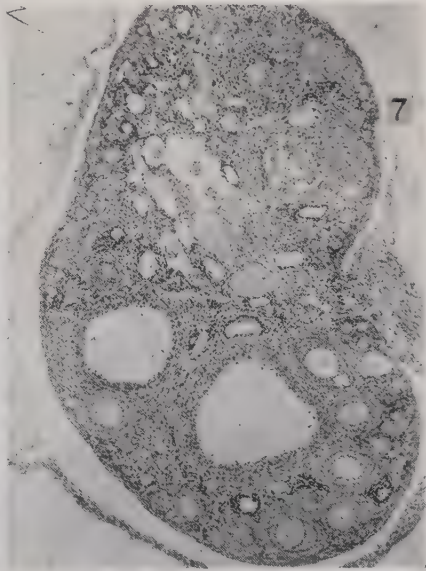
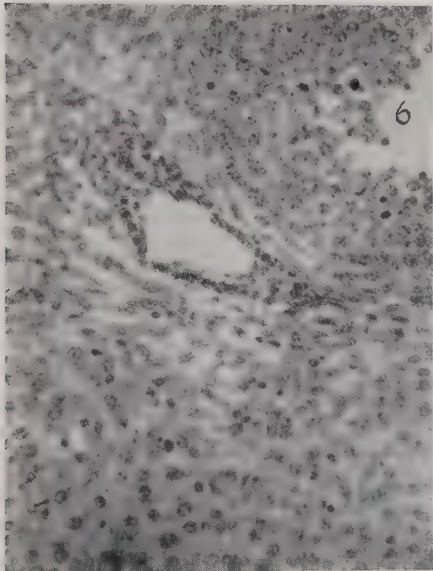
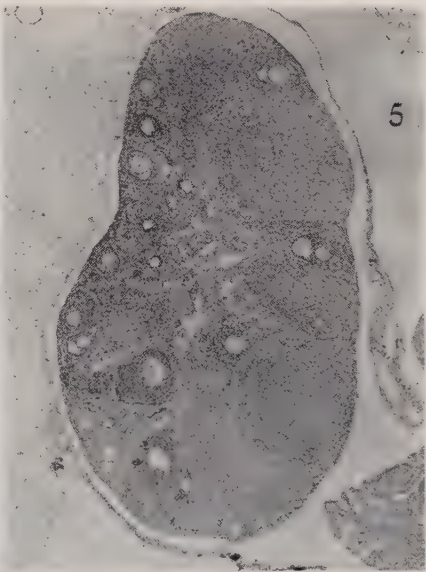
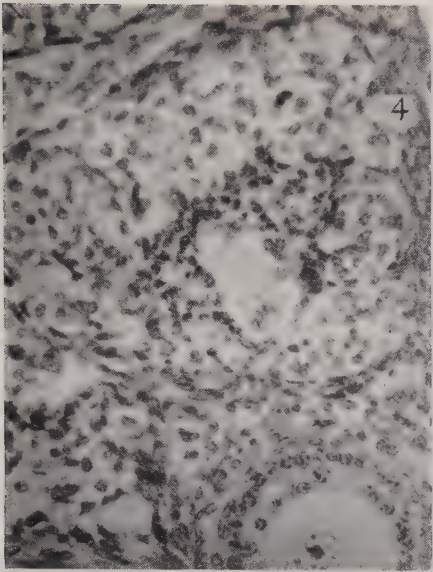


PLATE 2

EXPLANATION OF FIGURES

8 A field from the ovary pictured in figure 7. There are three normal follicles and two degenerating follicles whose granulosa cells are differentiating into wheel cells. $\times 310$.

9 A uterus of a control animal of the 1-month group. Note especially the height of the endometrial epithelium, the amount and loose appearance of the lamina propria and the large muscle cells of the myometrium. $\times 55$.

10 A uterus of a restricted animal of the 1-month group. There is no glandular development, the lamina propria is compact and the muscle cells of the myometrium are small with pointed nuclei. $\times 55$.

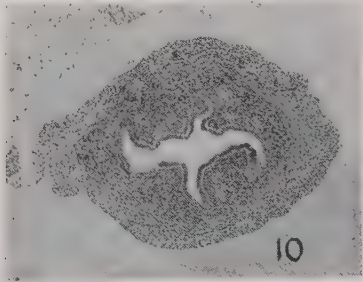
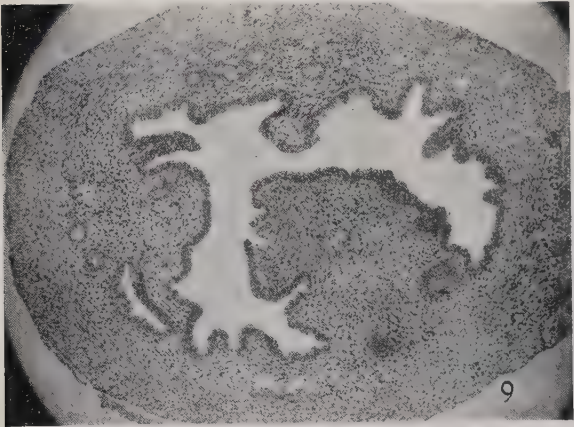
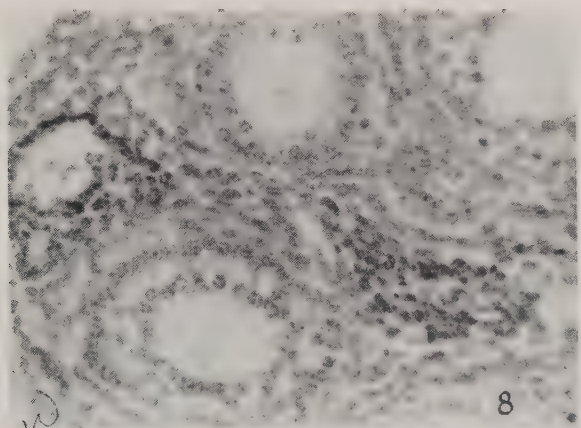
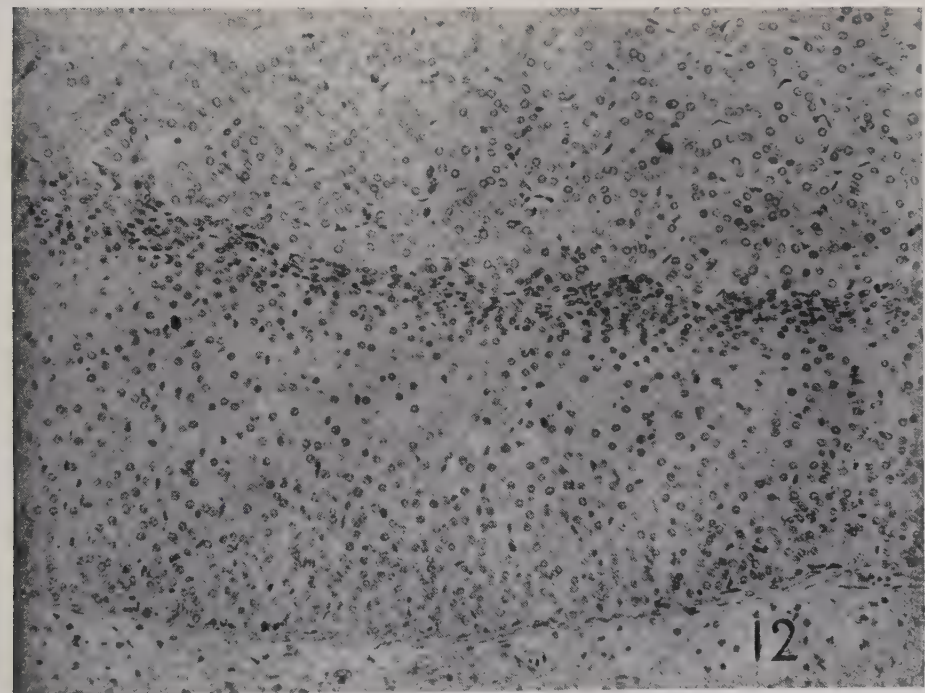
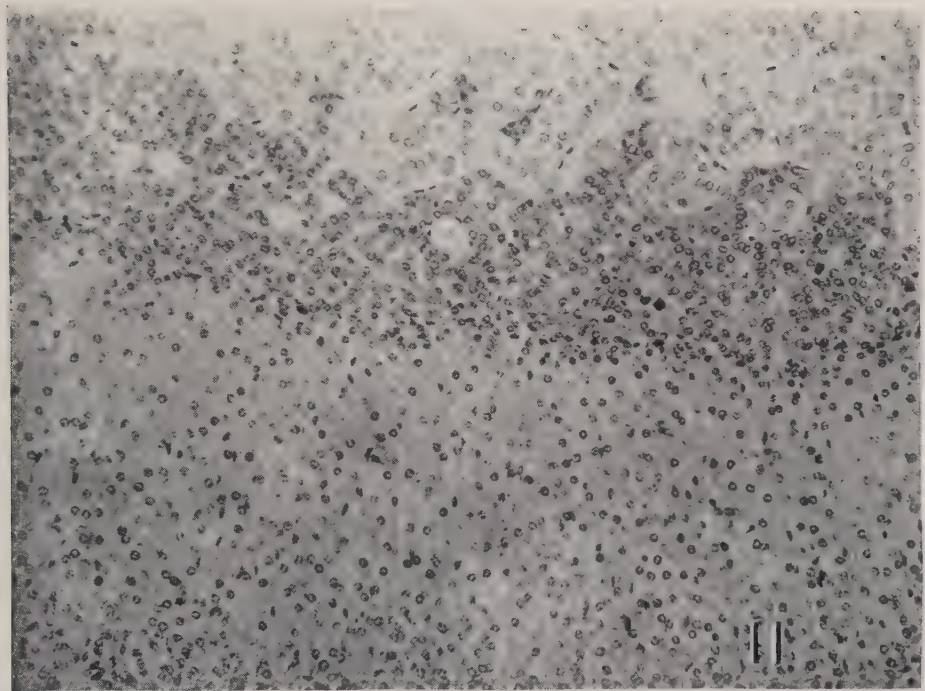


PLATE 3

EXPLANATION OF FIGURES

- 11 The adrenal cortex of a 1-month control mouse. Note the rather well developed X-zone containing a few large fat cells indicating the beginning of vacuolization. $\times 195$.
- 12 The adrenal cortex of a mouse after 1 month of diet restriction. The X-zone has been completely replaced by a layer of connective tissue. $\times 195$.



HYPOPHYSEAL CYSTS AND PITUITARY DYSTOPIA IN *TRITURUS VIRIDESCENS*¹

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ONE PLATE (FOUR FIGURES)

INTRODUCTION

In the course of an investigation of the morphology of the pituitary gland of *Triturus viridescens* in which 260 specimens were examined (Kent, '45) two instances of hypophyseal cysts and one instance of pituitary dystopia were observed. The occurrence of these abnormalities raises certain questions of pathological and phylogenetic importance.

HYPOPHYSEAL CYSTS

Two instances of epithelioid cysts were discovered, one of which was in the gland of a water phase newt, the other in a red eft. The cysts were of the same size and nature, and varied only as to their exact site with reference to the midline. That in the red eft was situated at the extreme lateral border of the gland between the pars distalis, and the pars intermedia (fig. 2). The cyst in the gland recovered from the water phase newt was situated in the midline and encroached on the pars distalis on the one hand and on the pars intermedia on the other, almost obliterating the pars intermedia in the midline (fig. 1). The cysts were spherical or nearly spherical, the left-right axis of the water phase cyst measuring 115 microns, the antero-posterior axis measuring 114 microns. The axes of the other cyst were a few microns shorter. The description of the cyst from the water phase animal will serve as representative of both the cysts encountered.

From its location, it is not possible to determine whether this is a cyst of the pars distalis or of the pars intermedia. It involves, however, chiefly the basophiles.

The lumen of the cyst is loosely filled with a detritus, most of which appears to be the remnants of cytoplasm of cells incorporated in the cyst as it grew. The detritus for the most part presents the appearance

¹ Contribution no. 70 from the Department of Zoology, Physiology and Entomology.

of a reticulum in which are found nuclei and cells in many stages of disintegration. Certain areas contain aggregations of small blue globules the origin of which is not known. These occur chiefly around the periphery, and may be disintegrating chromatin material. Aggregates of crystals are seen on occasion. In the cyst of the red eft an additional structure (not shown in the section illustrated) was seen in the lumen — a large, hollow, spherical globule of blue-staining material.

The periphery of the cyst is characterized by two important features: (1) The cyst wall appears in places to be of connective tissue origin; in other places it seems to be composed of low, ciliated epithelial cells. (2) Just external to this wall the cells show evidence of being necrotic, especially at the lateral borders of the cyst.

The cyst wall. At points on the circumference of the cyst the wall appears to consist of a condensation of connective tissue elements, of disintegrated cytoplasm, and of the nuclei of necrotic cells. This membrane stains faintly blue with Masson's stain, or, in many instances, is almost unstained. At other points, this membrane is replaced by cells which, in cross section, appear to be low cuboidal epithelial cells, or extremely high squamous cells. The nuclei are not always seen but occasionally a transected nucleus appears in the section. Long cilia, projecting into the lumen of the cyst, grow from the free borders of these cells. The basal bodies of these cilia are clearly visible and stain a deep blue with Masson's stain. The ciliated cells are limited to occasional areas along the cyst wall and do not form a continuous lining. The boundaries of contiguous epithelial cells are not generally visible except here and there. It is possible that these cells form a syncytium.

Rasmussen ('29) presents clearly the problem encountered in determining the presence of cilia. He states, "In several specimens striations in the colloid or in the shrinkage space next to the epithelium lining the residual lumen and related cavities were seen that suggested cilia but careful examination under oil immersion lens failed to show definitely that they actually were cilia." In other glands from the same form, however, he observed definite cilia. The same difficulty presented itself in the present investigation, and it was only after intensive investigation of the problem, and the submission of the material to colleagues who stated that they would not hesitate to say that cilia were present, that the writer asserts their presence in describing the cell wall. The hesitancy to describe a ciliated epithelium grew out of the difficulty in recognizing cell walls between the epithelial cells, and not in any defect on the part of the cilia which were in many cases clear, even to their basal bodies.

Necrotic tissue. Just external to the limiting membrane are found cells of two categories: differentiated granular and agranular basophiles, and necrotic cells. Necrosis is evident at all points on the periphery of the cyst. All stages in the process of necrosis may be observed in the cells surrounding the cyst. The cytoplasm becomes vacuolated, then disintegrates leaving a reticular cytoplasmic stroma around a glassy, shrunken, nucleus. The latter then breaks up and disappears and the remnants of the cell become part of the central detritus. While cells at tangential planes exhibit advanced disintegration, those farther removed show signs of becoming progressively involved, nuclear and cytoplasmic changes already having commenced.

DYSTOPIA

In the case of one red eft, the pituitary body was completely inverted, being located within the hypophyseal recess of the third ventricle and completely surrounded by the tissue of the hypothalamus and associated brain regions (fig. 3). In response to this condition, certain adaptations had been made both by the gland and by the surrounding tissue. These histological and anatomical adaptations will not be described in detail, but may be observed in figure 4. Among the changes was loss of arrangement of the cells in orderly columns typical of the normal gland. A pronounced basophilia was observed in the peripheral portion of the gland, where the latter was in contact with the brain tissue. Kingsbury and Roemer ('40) suggested, with reference to the pars intermedia of mammals, that "not only its presence as a distinct anatomic region, but also the histologic differentiation (or the lack of it) is an expression of the developmental relations, and not predetermined in a distinctive cell type." The pronounced basophilia exhibited about the periphery of the inverted gland constitutes evidence in support of this suggestion. The cells along this peripheral region were oriented with long axes parallel to the border of the gland. This condition seems to constitute an excellent example of the result of pressure on the orientation of cells in a cell mass. The relative number of acidophiles present in the gland was small, and those present were chiefly of the non-granular type. There was a large increase in the number of adipose cells and in the size of these cells in the meninges about the brain, a condition which compels attention when the brain is examined histologically.

DISCUSSION

Cysts. Hypophyseal cysts are not uncommon among the birds and mammals. An incidence of 10% is reported by Chadwick ('36) for the

guinea pig, and by Oppen ('40) for the rat. Cysts occur less frequently in the glands of the rabbit (Trautmann, '09), opossum (Martins, '33), cattle (Dostoiewsky, 1886), and in the human hypophysis (Rasmussen, '28; Frazier and Alpers, '34). Cysts were reported in the domestic fowl by Collin ('26), and by Rahn ('39) the latter investigator reporting cysts in almost every gland examined.²

Copeland ('43) reported five cysts from 300 specimens of *Triturus viridescens*, the only instances of hypophyseal cysts among amphibia that have been encountered in the literature. Among the reptiles, cysts have been reported from at least two genera, *Anolis carolinensis* (Poris, '41, one cyst only), and *Sceloporus undulatus* (Altland, '39, one cyst in 200 glands).

Difficulty is encountered in compiling a bibliography of papers reporting hypophyseal cysts, since in many cases the reference is incidental, and no hint is given in the title as to whether or not cysts are reported. The list of forms below the mammals in which cysts have been reported is not extensive.

Nearly all cysts reported in the suprasellar area of mammalian glands exhibit ciliated epithelium in the walls, according to Frazier and Alpers ('34), who class such cysts as "true tumors of Rathke's cleft." The cyst reported from *Anolis carolinensis* was regarded by Poris as "an embryological rest originating from Rathke's pouch." The cysts of the chick as reported by Rahn were "ciliated colloid cysts," and were reported to have no connection with the residual lumen.

The presence of cysts in the amphibian gland is of pathological and phylogenetic significance in view of the mode of development of the amphibian pituitary gland. No Rathke's pouch is formed; no residual lumen secondarily appears. Both of the cysts reported in the present paper, however, were located at the immediate boundary between the *pars intermedia* and the *pars distalis* (the site of Rathke's cleft when residual). The facts therefore suggest two possible interpretations: It is possible that, although no Rathke's cleft secondarily appears in the Amphibia, the cells of this region may be genetically capable of producing such a lumen or, as Copeland says, may have a latent lumen-forming tendency. When such a lumen is formed, the result may be the production of a large cyst, accompanied by a metaplasia of certain cells between the *pars distalis* and the basophilia bed, and necrosis of some of the elements. The other interpretation suggested is that not all cysts found in the region between the *pars anterior* and the *pars intermedia* are necessarily due to the arrested development of embryonic rem-

² For an extensive bibliography the works cited may be consulted.

nants of Rathke's pouch. They may possibly have a pathological origin other than from a pre-existing cavity, or potential cavity. This interpretation is strengthened by the presence of widespread necrosis. Such a conclusion is suggested in view of the studies of Rahn ('39), with reference to the chick hypophysis.

The incidence of cysts is somewhat greater than 1% in *Triturus*.

Dystopia. It is an interesting commentary that, when the animal exhibiting pituitary inversion was killed, the technician deviated from usual procedure in making a special note of its small size, in addition to recording the actual measurements. The smallness of the animal is referable possibly to the noted basophilia, and may constitute a condition opposite to that found in acidophile cell tumors, which are accompanied by acromegaly. The increased number and size of the adipose cells may be a related phenomenon. "Neighbor pressure-effects" (Cameron, '35), due to the dystopia, may also have depressed pituitary activity.

The inversion exhibited by the pituitary body in this animal produced abnormalities parallel to those often described in man and which, taken together, constitute Fröhlich's syndrome. Such parallels between dysfunction of the pituitary body in mammals, and in vertebrates as far down the phylogenetic scale as the Amphibia seem to emphasize, succinctly, the strong genetic ties between man and the lower vertebrates.

SUMMARY

1. Two instances of hypophyseal cysts encountered in *Triturus viridescens* are reported and described. Both cysts occurred in the region between the pars anterior and the pars intermedia.

2. Hypophyseal cysts occurring between the pars anterior and the pars intermedia in other vertebrates are often ascribed to remnants of the residual lumens. No residual lumen occurs in *Triturus*.

3. An instance of pituitary dystopia in *Triturus* is described wherein the gland of one red eft was located entirely within the hypophyseal recess of the third ventricle. The animal exhibiting this condition was extremely small and exhibited a pronounced basophilia, particularly in the peripheral portions of the gland. An unusually large number of adipose cells were observed in the meninges about the brain of this animal.

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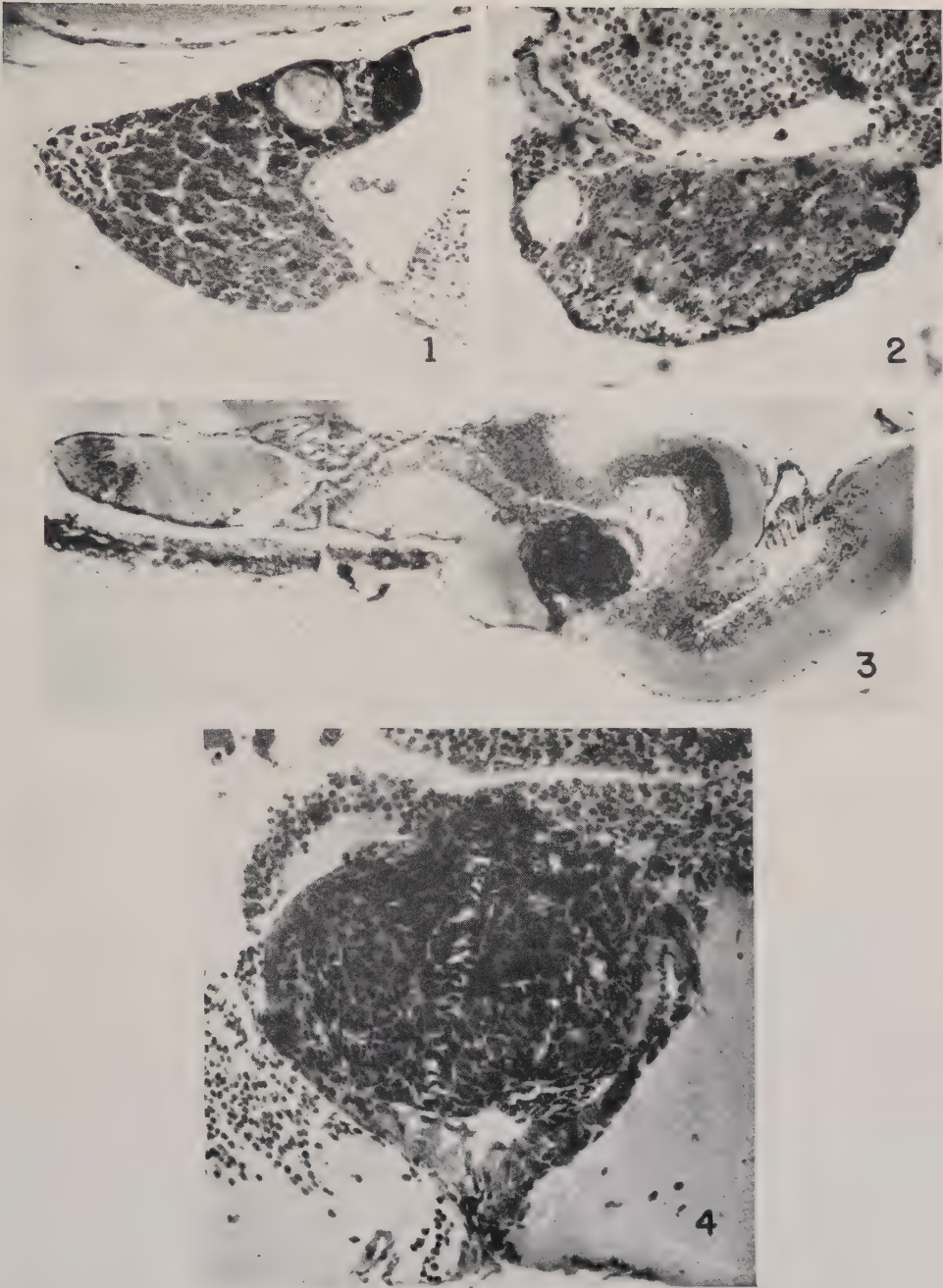
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PLATE 1

EXPLANATION OF FIGURES

- 1 Median sagittal section of hypophysis from water phase newt, anterior end to the right, showing cyst. $\times 90$.
- 2 Transverse section of hypophysis from red eft showing cyst. $\times 90$.
- 3 Sagittal section of entire brain of a small red eft, anterior end to the left, showing inverted pituitary occupying hypophyseal recess and surrounded by nervous tissue of the hypothalamus. $\times 30$.
- 4 Enlarged view of inverted pituitary. $\times 100$.



NOTES ON METHOD FOR REPRODUCING CROSS SECTIONS OF THE BRAIN AND SPINAL CORD

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ONE FIGURE

A satisfactory method for demonstrating brain cross sections in the neuroanatomy laboratory sometimes poses a problem. To properly study mounted sections some form of "viewing box" is necessary to give sufficient illumination and is satisfactory only for a small group of students. A preparation of a serial section of a brain represents considerable time besides representing valuable, breakable, and not easily replaceable collections for study. Therefore an inexpensive, quick, and achromatically correct reproduction of such sections would be highly desirable.

Because of its simplicity and uniformly good results, the Weigert myelin stain is employed (for this method see reference). The myelinated fiber tracts are deeply stained and the nuclei are unstained, thus causing a reversal from the previous appearance of white and gray matter. If any confusion arises when viewing sections stained in this manner, it is due to difficulty in correlating prepared sections with fresh material undergoing dissection. Sections are cut at 100–150 μ

To reproduce cross sections of the brain and spinal cord care should be taken that the sections prepared by the Weigert method are well stained and are not too pale, since the Weigert sections themselves will be used as photographic negatives and should give good differentiation. No camera, lenses, film or developing equipment is necessary. All that is required is a standard photographic printing machine or enlarger of sufficient size to accommodate the section employed as the negative. From this section all contact prints or enlargements are made.

Two methods of mounting the sections will be described. To make contact prints employing a minimum amount of photographic equipment, it is necessary that the section be very close to the printing paper. This means that as thin a cover slip as possible must be used. Sheets of a sufficient size and of the thickness of a no. 3 coverslip should be obtained. The section is mounted with a clear medium clarite on a $\frac{1}{16}$ "

glass plate. The section must be perfectly flat and have no air bubbles. The coverslip is then applied and gently pressed down. When dry it will be satisfactory for making contact prints, the printing being done with the thin side toward the paper. However, if an enlarger (Eastman Projection Enlarger) is to be used, a more satisfactory method consists of mounting the sections between two $\frac{1}{16}$ " glass plates. This is easier to do and affords a more substantial preparation. When dry such a section can be put into the enlarger, the image brought into

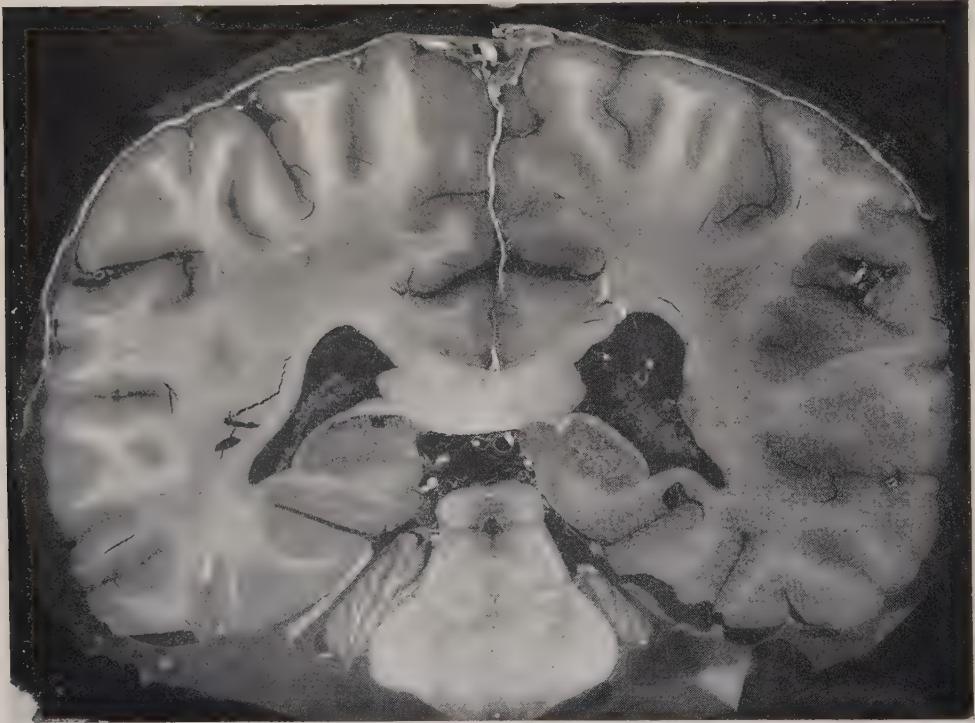


Fig. 1 Cross section of brain.

sharp focus and then printed in the usual manner (fig. 1). Enlargements are limited only by the size of the enlarger, but have proved very successful with as high as a six-fold enlargement.

Contact prints can be made from thick cover preparations, but very little detail will appear because of light diffusion in the glass between the tissue and the paper. The use of polarized light does not eliminate this. Good results are obtained by printing on no. 4 Kodabromide glossy paper. Lighting details are left to the discretion of the technician. If

equipment is not readily available, any commercial photo-finishing establishment will be able to make the prints or enlargements.

To summarize, this method provides an inexpensive way of reproducing in quantity cross sections of the brain and spinal cord, either with their actual size or with moderate magnification. The reproductions correspond to the unstained brain and are not "negatives" as are the original Weigert myelin stained sections. In other words, the original appearance of the unstained section will correlate with the photograph, nuclei and gray matter appearing darker than white matter. No special equipment is necessary for viewing the sections; neither is camera or film necessary for their production. Sections may be enlarged as many as six times with good results. The sections may be labeled and marked; they are not broken if dropped and their loss is of no consequence. A few strategic sections will make an inexpensive and permanent atlas for the student of neuroanatomy.

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THE POLYMORPHONUCLEAR CELL AND ITS OCCURRENCE WITHIN THE LARYNX OF THE CAT¹

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TWO PLATES (TWELVE FIGURES)

INTRODUCTION

Within the larynx of the cat, in lateral folds which may be descriptively termed 'ary-epiglottic folds' (aryteno-epiglottic plicae), at about 2 weeks after birth there is developed a close aggregation of characteristic lymphatic nodules dignified by the name of 'laryngeal tonsils' (Kingsbury, '43). The proliferative growth that establishes within the larynx this area of dense lymphatic tissue does not seem to be exclusively that characteristic of lymphocyte formation. The stroma of the fold, particularly in the earlier stages of the growth, is often rich in free cells suggestive of the formation of other cell types. This is not, however, a unique observation. For, in the development of the pharyngeal tonsil of the cat it has been shown that "in the zone between the developing lymphatic tissue and the fibrous capsule, appear in later fetal life: (a) large blastic cells; (b) "monocytoid" and transitional cells; (c) "myeloid" cells; (d) neutrophiles; (e) eosinophiles; (f) erythroblasts and an erythropoiesis" (Kingsbury, '32, p. 293). Likewise in the development of the human pharyngeal tonsil, it has been reported that "in the region bordering the tonsillar area" myelopoiesis and erythropoiesis are encountered. "Within these areas large numbers of eosinophilic and neutrophilic myelocytes and lymphocytes are formed" [and here are] "regions of erythropoiesis" (Snook, '34, p. 33). In the development of the palatine tonsil of the cat, are also encountered myeloblasts and myelocytes, granulocytes, and "cells of the erythropoietic series" (Ramsay, '35, p. 189). Latta ('21) had earlier described in the rabbit granulopoiesis and erythropoiesis bordering the dense lymphatic tissue of the intestine (Peyer's patch). Doubtless other similar cases have been recorded. Jordan ('29) in a number of papers has described in the rabbit erythropoiesis within lymph nodes. No granulopoiesis was noted as there occurring.

¹ This study was made in the Department of Anatomy of the University of North Carolina. The writer wishes to acknowledge with appreciation the facilities so fully placed at his disposal.

Such instances of the formation of cells of the myeloid type in intimate association with developing lymphatic tissue can hardly be described as "extramedullary myelopoiesis" nor as "myeloid metaplasia". They have, however, a value in supporting the unitarian concept of blood cell formation and may be of significance in the interpretation of the cell types themselves.

Within the ary-epiglottic folds of the cat the determination of the cell types which are encountered is not as readily made as is the case, for example, in the zone bordering the developing pharyngeal tonsil. This is due in part to the density and to the typically rich content of free cells in the early weeks after birth. There is no evidence of a local formation of eosinophiles, nor does an erythropoiesis occur, although it was noted in a single instance in the thyro-arytenoid fold. There are found, however, in the ary-epiglottic fold, numerous polymorphonuclear cells which seem to be of two types: (1) derivatives of cells of the lymphocytic series which are interpreted as forms of degenerative change; (2) cells indistinguishable from the polymorphonuclear neutrophils of the blood.

As the ary-epiglottic fold during this period — namely, the first 2 or 3 weeks after birth — is not only rich in free cells but also usually quite vascular, it is of course possible that at least some of the cells of the second type within the fold, in the covering epithelium or on the surface, are from the blood. This cannot be directly determined. It is more than possible that a formation of polymorphonuclear cells of this type occurs locally. In certain regions within the larynx of the cat such a local origin is evident. It appeared worthwhile therefore to present in detail the evidence, and twelve series through the larynx² were specially studied. The polymorphonuclear neutrophile of the blood is admittedly a peculiar cell type. Of necessity its interpretation and the cell changes which produce it are considered.

OBSERVATIONS

As has been previously stated, the ary-epiglottic folds of the cat's larynx, before and during the formation of the tonsillar tissue are rich

² The larynx series, covering roughly the first 6 weeks of postnatal life, which were selected for critical examination, are listed below. Fixation was in Bouin's fluid, Zenker's fluid, or Maximow's Zenker-formol, four series each. Staining was in hematoxylin and eosin in all save two where azurephloxine neutral stain was utilized. In the last four series measurements are from occipital crest to root of tail.

(1) fetus, 130-mm. length; (2) fetus, near term, 150 mm.; (3) new born; (4) kitten of 155-mm. length; (5) 2 days postpartum; (6) 13 days postpartum; (7) 16 days postpartum; (8) 32 days postpartum; (9) 17.0 cm.; (10) 18.0 cm.; (11) 19.0 cm.; (12) 20.5 cm.

in free cells, and while polymorphonuclear cells occur, they are so scattered that adequate presentation of evidence as to their source would involve an excessive wealth of illustration. While the content of the ary-epiglottic fold has in each instance been carefully examined, but two figures from one stage have been selected in illustration (figs 1, 2). Elsewhere within the larynx, however, were encountered two areas of marked and seemingly exclusive formation of polymorphonuclear cells. From these the main illustration is drawn and presented as evidence of a local origin of cells of this type within the larynx.

The first of these areas is from the first of the series, where near the caudo-lateral edge of the thyro-arytenoid fold an area of polymorphonuclear cells, cells with horse-shoe-shaped (U-shaped) nuclei, transitionals and a number of "blastic" cells are found in close formation. The area extends for fifty-three sections (of 10μ). It lies mainly lateral to the arytenoid cartilage and ventral to it. It is not markedly vascular; the blood vessels being few, small and not congested. The connective tissue is loose (reticular?) but denser bordering the deeper side of the area which is thus essentially within the mucous membrane. A second small area of mononuclear cells (only) with mitosis lies within the "central core"³ of loose connective tissue (fig. 5) near the arytenoid cartilage.

Four photographs (figs. 3, 4, 7, 8) from the central region of the first area are introduced to illustrate the presence of (a) large blastic cells (figs. 4, 7), (b) smaller mononuclear cells and cells with transitional nuclei (figs. 3, 8), (c) cells with U-shaped nuclei (figs. 3, 7, 8), and (d) definitive polymorphonuclear cells (figs. 4, 7). The evidence seems conclusive for the formation of typical polymorphonuclear cells from cells with a U-shaped nucleus which in turn are derived from the mononuclears through a change in nuclear form.

Such apparently fortuitous areas of free cell formation are not ideal for cytologic determinations, since it is impossible to determine in advance where they will be encountered. The application of special techniques, therefore, becomes difficult or impossible. Thus, little detailed information as to the cytoplasm was gained. A centrosphere and centrioles were not identified. It can only be said that the cytoplasm of the cells — polymorphonuclear cells, monocytoid cells and some at least of the mononuclear cells — was granular, but the distinctive character of the granules was not determined.

³ Comment on this area of connective tissue was earlier made (Kingsbury, '43, p. 176, footnote 2, and on p. 182).

The series in which area 1 was found was cut in the sagittal plane and included but half the larynx. It was therefore impossible to determine whether a comparable area of polymorphonuclear cell formation occurred in the other (left) thyro-arytenoid fold of which only the median edge was included. In the last slide of the series, however, within the core of loose connective tissue previously referred to, there occur on the left side groups of larger blastic cells (fig. 6) and also a larger group of smaller mononuclear cells. These suggest a proliferative activity of some type.

In the second series and in the same general region as group 1, i.e., adjacent to the arytenoid cartilage, a much smaller group of polymorphonuclear cells was found. It was present, however, on one side only as was easily determined since the series was in the transverse plane.

The second area to be considered is found in series 10, from an older kitten. Photographs from this area were earlier made for record and were reproduced (Kingsbury, '43, figs. 19, 20). This area lies in the region ventro-lateral to the base of the epiglottis and the cephalic end of the ary-epiglottic fold. It borders the ventral end of the thyro-arytenoid fold and is adjacent to the sympathetic ganglion — which it partially surrounds — and to the branches of the superior laryngeal nerve found in this locality (Kingsbury, '43, p. 180). The area is not extensive, being limited to twenty-four sections (of 10μ); it is not complete, however, since the series does not extend beyond the cephalic end of the ary-epiglottic fold. The connective tissue is denser medially, looser laterally, particularly beyond the area where scattered large blastic mononuclear cells occur including large mononuclear eosinophiles with an occasional polymorphonuclear cell. The vascularity of the area is slight and congestion is not apparent. Lymphatic channels, large and small, border the area.

Figures 9, 10, 11 and 12 from the more caudal, intermediate and cephalic regions of the area are offered in illustration. The larger blastic cells (figs. 11, 12) are in general more lateral in location, the polymorphonuclear cells are concentrated more medially. There may be noted the large number of cells with U-shaped nuclei which vary somewhat in size. It is not difficult to find, on the one hand, intergradations between these cells and the mononuclear cells and, on the other hand, transitionals with the more distinctive polymorphonuclear cells. For the reasons stated in the case of group 1, it is impossible to determine the presence of a centrosphere (and centrioles), nor to es-

tablish the nature of the granulations which occur and which may be seen in some of the photographs.

The series was limited to one half of the larynx, hence it could not be ascertained whether or not a comparable area was present on the opposite side. On the same side a small group of blastic mononuclear cells occurs in the cephalic edge of the thyro-arytenoid fold which in its superficial zone is quite cell rich and vascular, as is also the neighboring ary-epiglottic fold. The latter contains many polymorphonuclear cells, in the stroma, in veins, epithelium and on the surface where they were figured in an earlier publication (Kingsbury, '43, fig. 13).

In series 11 a much smaller area of blastic mononuclears and polymorphonuclears occurs in the same general region. This series is also sagittal and includes but half of the larynx so again conditions on the opposite side could not be determined. No other marked areas of formation of polymorphonuclear cells were found in the other series examined.

It has already been noted that in the ary-epiglottic fold, preceding and at the time of appearance of the laryngeal tonsil, the connective tissue of the fold typically becomes rich in free cells including blastic cells and frequently polymorphonuclear cells. Two figures were then referred to (figs. 1, 2). The difficulties of analysis in the rather diffuse representation of stages were then emphasized. If they are compared with the figures from groups 1 and 2, however, it will be seen that a few polymorphonuclear cells, cells with U-shaped nuclei, mononuclear cells (and lymphocytes) are present.

It may also be noted that in the loose connective tissue of the thyro-arytenoid fold (cf., Kingsbury, '43, footnote 2) groups of blastic cells are to be expected occasionally, as in series 1 and again in series 5 (Kingsbury, '43, fig. 21). In the latter series in addition a small area of low-grade erythropoiesis occurs as was recorded (Kingsbury, '43, fig. 22). This was an exceptional observation. No polymorphonuclear cells were seen in this location in this series.

DISCUSSION

In reviews dealing with the polymorphonuclear neutrophile Bunting ('32, '38) makes the following comments: "Except for their presence in the bone marrow, neutrophile leucocytes appear to be confined to the blood stream in the normal man or animal"; and "that which distinguishes the neutrophile morphologically from all other body cells is the character of the nucleus". These statements but reflect the generally accepted conclusions of the hematologist and clinician. In view of the observations offered in this paper, however, they present the sug-

gestion of a dilemma. Much attention has been paid to the origin of the neutrophile within the bone marrow, but the seriation of stages from "myeloblast" to definitive leucocyte is of descriptive value only, whether stages are termed myelocytes A, B, and C, or promyelocyte, myelocyte and metamyelocyte. The basic problem of the cytologic changes involved and the fundamental cell physiology underlying the transformations are less considered. Two investigators occur to me, however, as having made significant contributions; Schilling and Jordan.

Schilling's interest in the blood, primarily clinical, led to a critical consideration of the Arneth "shift to the left", that is, the appearance in the blood in certain conditions, of immature forms and/or myelocytes. He was thus led to consider the character of the changes leading to the "mature" neutrophile. Under the dark-field microscope ('08) he was able to identify the centrosphere (which he variously designated as centrosome, central apparatus, archiplasmic mass). To a swelling of this he seemingly attributed the transformation of the myelocytic nucleus to the elongated and U-shaped nucleus of the metamyelocyte and the young neutrophile ('22), using as illustration the analogous nuclear change of shape described by Ballowitz ('00) in the cells of the Decemet membrane of the cat. Both nucleus and centrosphere Schilling believed to be passive in this transformation. In the amoeboid movement of the neutrophile, however, he thought the centrosphere played an essential role with the nucleus remaining passive throughout. Centrioles (centrosomes) within the centrosphere were apparently not observed by him. The division of the nuclear strand ("Stab" nucleus) into segments, 2 to 5 in number in man, was interpreted as progressive (Brugsch and Schilling, '08) but not a record of the age of the neutrophile and probably influenced by the amoeboid movement.

It may be doubted that the relation of nucleus and centrosphere in the transformation to the polymorphonuclear neutrophile is as crudely mechanical as Schilling seemed to infer. In the present writer's opinion there are reasons for regarding the centrosphere (cytocytrium) as the metabolic center of the cytoplasm and that according to such evidence as there is, the nucleus plays a significant part in the cell (cytoplasmic) metabolism. There seems to be, however, no evidence that the segmented nucleus is other than passive. Furthermore, as Schilling ('20, '22) later seemed to recognize, the effect of amoeboid movement upon the segmentation of the Stab nucleus appears insignificant or questionable. Schilling at that time concluded that polymorphism is a general peculiarity of the nucleus of the connective tissue cell, but only in the neutro-

phile is it specially expressed and clear. His further statement seems of particular significance: that the division of the large nucleoli of the young neutrophile into several smaller nucleoli either accompanies or parallels the segmentation of the Stab nucleus. This fragmentation of nucleoli, he states, is always to be observed.

Jordan ('19) in an analytical study of the blood of the frog has made a suggestive contribution to the cytological interpretation of the neutrophile which, in the writer's opinion, might well be extended to other animal forms. The "resemblance to the polymorphonuclear neutrophile leucocytes of mammals is striking". The attraction sphere (centrosphere) was conspicuous; generally situated within the concavity of the lobulated nucleus. Within the centrosphere in cells with less complex nuclei are found a centrosome or diplosome; in cells with a more complex polymorphous nucleus the centrosome(s) are replaced by a mass of minute granules, the inference being that the later were derived from the centrosomes by a "disintegration or partition". The specific cytoplasmic granules first appear near the centrosome and "spread in radiating fashion toward the periphery of the cell", a disposition maintained in the definitive leucocyte. Only the young neutrophile with round or oval nucleus divides by mitosis; older cells Jordan believed may divide by amitosis and he poses the query whether the lobulation of the definitive leucocyte may not express an incompleting amitosis.

It may be noted that Jordan found that lymphocytes circulating within the blood of the frog directly transformed to polymorphonuclear cells. Downey ('13) had earlier made the same statement for the salamander (*Amblystoma*). There seems, however, to be a question as to whether such polymorphonuclear cells of the amphibian blood may be directly homologized with the mammalian neutrophile; there is involved the question of the specific value of the staining reactions of the granules. These differ in staining in the two groups.

Jordan does not so interpret but his observations offer the suggestion of an altered nucleo-cytoplasmic relation with a degenerative trend in both nucleus and cytoplasm. Whether the appearance of the cytoplasmic granules should be so included is not clear. The interpretation of the observations made the subject of the present discussion may possibly on such a basis be intelligible without too close a comparison with the neutrophile leucocyte of the blood. Furthermore, there is indicated a consideration of the cytological relations in other cells of the hematological series, for in all a close association of nucleus and cyto-centrum is present. Utilizing freely the reviews of Maximow ('32a,

32b) the salient morphological features of the nucleus-cytoplasmic relation may be mentioned.

In the small lymphocyte two centrioles occur at the indentation of the nucleus, but no cytocentrum is demonstrable. In the large lymphocyte, the plasma cell, the histiocyte and macrophage, two centrioles are present within a cytocentrum next the nucleus. It is, however, only in the monocyte, that difficult cell⁴ for the hematologists, that conditions are found paralleling the early stages of formation of the polymorphonuclear neutrophile leucocyte. In the typical monocyte, the nucleus is greatly indented, with the cytocentrum within the cytoplasmic bay. The morphology is suggestive of an altered nucleus-cytoplasmic relation. In the more extreme forms with horse-shoe-shaped or U-shaped nuclei the suggestion is even stronger. Such cells are numerous in the areas considered in this article. Furthermore, if one accepts the hypothesis of an altered nucleo-cytoplasmic relation, it becomes probable that under certain conditions, blastic cells of any character (whether lymphoblastic, leucoblastic, or other formative cells may yield cells with U-shaped or even segmented nuclei, which, while polymorphonuclear, need not for that reason be directly placed in the same category with the polymorphonuclear neutrophiles of the blood. The fundamental problem is clearly that of the metabolism and physiology of the cell. It may be suggested that, in general too great emphasis is usually placed upon definite and distinctive cell types and that the teleological conception of function is excessively dominant.

In conclusion, two statements may be made concerning the two areas from the larynx of the cat more specifically illustrated in this article: (1) The proliferative reactions described in no way conform to those characteristic of an inflammatory reaction, unless indeed, local proliferative reactions leading to the formation of polymorphonuclear

⁴ Maximow ('32b, p. 748) characterizes the monocyte as follows: "The monocytes are neither a lymphoid nor a myeloid cell, nor do they represent a peculiar specific cell type as claimed by the triallistic theory. They, for the most part, are peculiar stages of development of the ubiquitous lymphoid cells. Their development is not confined to a certain tissue or organ . . . they are incapable of hematopoietic activity and their prospective potencies of development seem to be restricted to the same extent as those of the histiocytes".

The following comments on the monocyte are made by a "trialist", Schilling, ('29, p. 133): "Segmentation and sausage-like formation [of the nucleus] (very similar to young neutrophils) are seen at times as a pathological condition. The large forms with lobulated nuclei are still called 'transitional' forms since they were formerly considered transitions from neutrophils to lymphocytes. . . . Certain primary forms, leucoblasts, promyelocytes and lymphoblasts, when they enter the blood under pathologic conditions, show a monocytoïd appearance, making it difficult to classify them ('atypic monocytes'). . . . Those cells alone should be considered monocytes which have an absolute resemblance to normal types; all doubtful elements should be considered as lymphocytes."

cells occur in inflammations more constantly or more frequently than is suspected. The generally accepted doctrine is, of course, that polymorphonuclear cells found in the connective tissue are derived exclusively from the blood. Since in the larynx the presence of polymorphonuclear cells in most cases occurs after birth, the existence of a local infection might be suspected. This is not true in the case of group 1 which was antenatal, as were also several of the other instances mentioned in the introductory paragraphs of this communication. No suggestions can be offered as to the causative factors operative in the larynx of the cat.

(2) There is no reason to suspect that such areas of proliferation and transformation as are here described are peculiar to the cat. On the contrary, there is every reason to suspect that if abundant material from the larynx of other mammals were examined similar centers might be encountered. Doubtless, also, there are many other localities where a certain degree of atypical proliferation may occasionally be found. Places where such ectopic granulopoiesis or erythropoiesis is encountered in varying degree seem to frequently border regions of lymphopoiesis, that is, lymphatic tissue.

SUMMARY

1. There are described in some detail two areas within the larynx of the cat where the formation of polymorphonuclear cells is found. They are derived from cells with U-shaped nuclei which in turn are derived from blastic cells.

2. The comparison of such polymorphonuclear cells with the polymorphonuclear neutrophiles of the blood is considered, and

3. The cytological changes of the "myelocyte" to the neutrophile leucocyte are discussed.

4. The cell with the U-shaped nucleus is compared with the typical monocyte.

5. The suggestion is made that from the standpoint of cell physiology, both monocytes (including monocytoïd cells) and polymorphonuclear cells express in their morphology a degenerative trend.

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PLATE 1

EXPLANATION OF FIGURES

The magnification of all figures is $\times 660$.

1 and 2 Larynx of kitten 16 days post partum. Bouin's fluid, hematoxylin and eosin, 10 μ . Cell types from the stroma of the ary-epiglottic fold; mononuclear cells, cells with U-shaped nuclei, polymorphonuclear cells, lymphocytes, etc.

3 and 4 Larynx of fetal kitten, 130 mm. Bouin's fluid, hematoxylin and eosin, 10 μ . From the connective tissue of the mucous membrane of the thyro-arytenoid fold, "area 1": blastic cells, transitionals, cells with U-shaped nuclei, polymorphonuclear cells, etc.

5 and 6 Same specimen as that in figures 3 and 4, showing groups of mononuclear cells from the connective tissue "core" of the thyro-arytenoid fold. Figure 5, a group of smaller cells from the right side; figure 6, larger blastic cells from the left side.

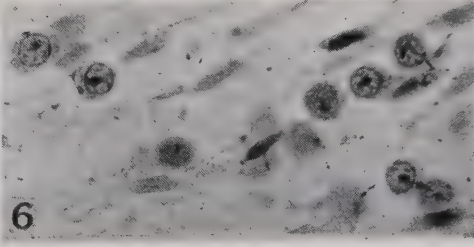
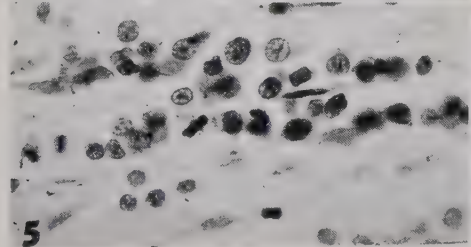
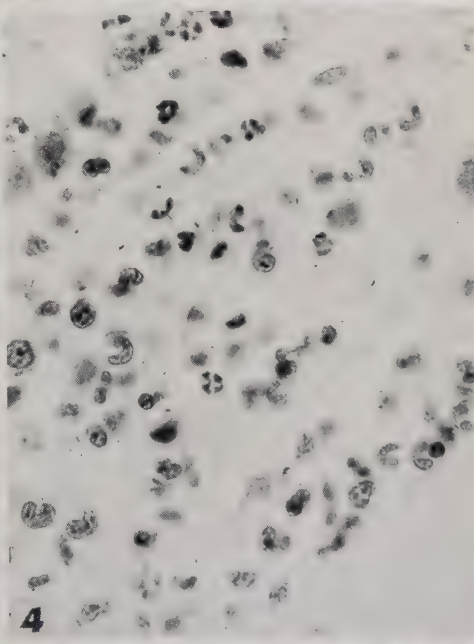
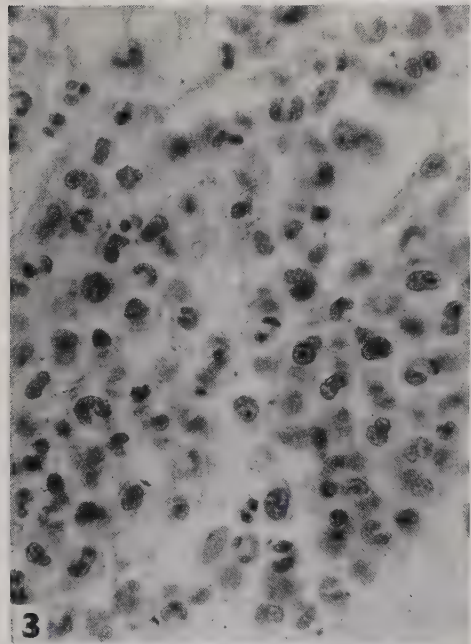
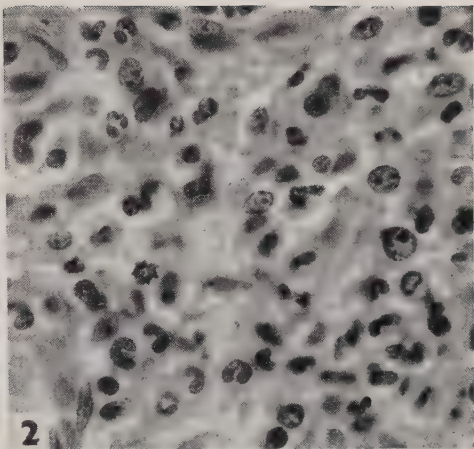
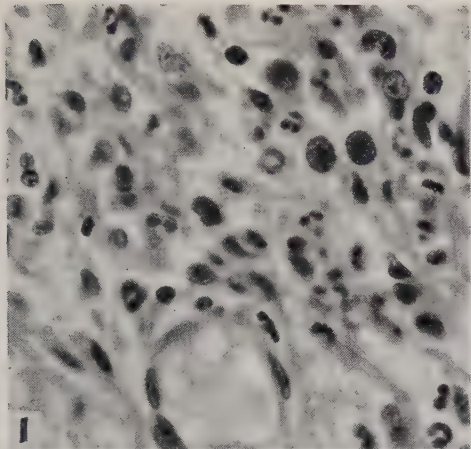


PLATE 2

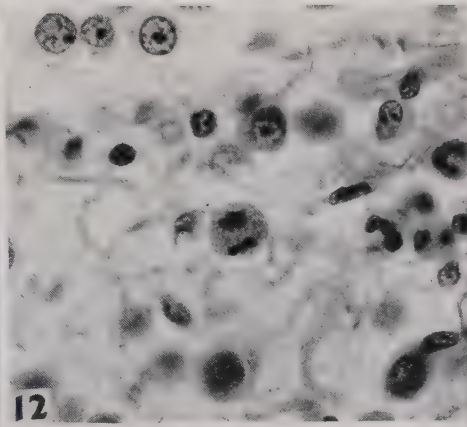
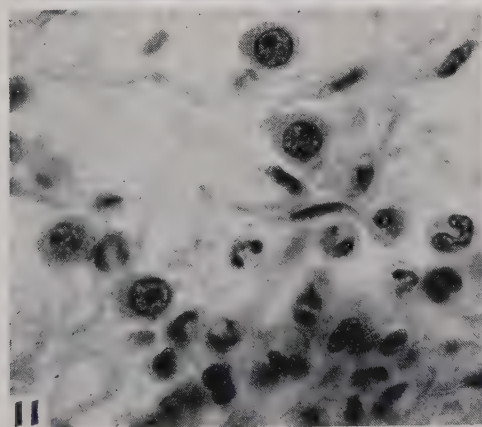
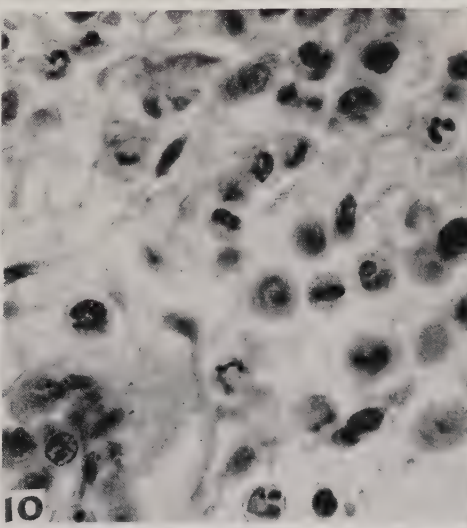
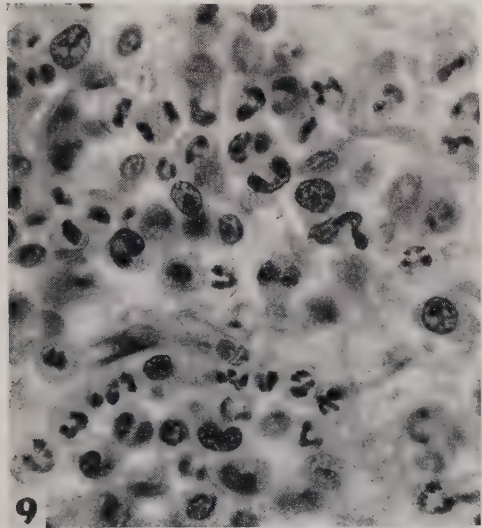
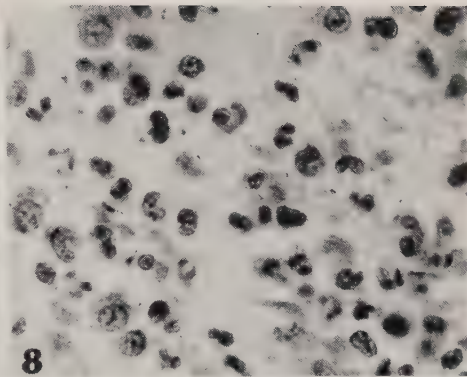
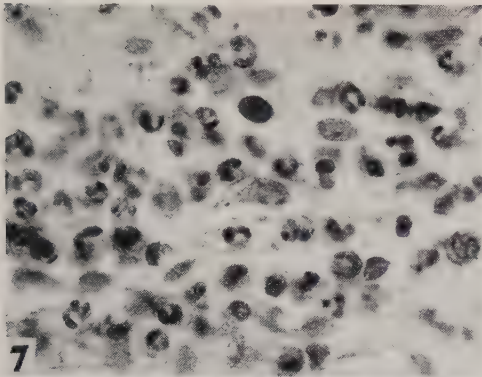
EXPLANATION OF FIGURES

The magnification of all figures is $\times 660$.

7 and 8 As in figures 3 and 4. From area 1. To illustrate particularly mononuclear cells, cells with U-shaped nuclei, polymorphonuclear cells, transitionals.

9 and 10 Larynx kitten, 18.0 cm. (occipital crest to root of tail). Zenker's fluid, hematoxylin and eosin, 10μ . To illustrate the cell types found in area 2, mononuclear cells, cells with U-shaped nuclei, polymorphonuclear cells, transitionals. A mitotic figure occurs in figure 9.

11 and 12 As in figures 9, 10. To illustrate particularly the large and medium blastic cells. A cell in figure 12 is in mitosis.



THE COMMON OCCURRENCE OF "TESTIS" CORDS IN THE OVARIES OF A SHREW (*SOREX VAGRANS*, BAIRD)¹

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TWO PLATES (TWELVE FIGURES)

In the course of studies on the comparative anatomy of the mammalian ovary it was observed that the ovary of *Sorex vagrans*, Baird, a common western shrew, frequently contained unusual and conspicuous structures which resembled immature testis tubules. The fact that they were found in several individuals indicated that they were probably normal for this species, and that we were not dealing with a relatively rare hermaphroditic condition or ovotestis. We therefore at first assumed that the structures were exceptionally well developed medullary cords, and decided to make a survey of their occurrence and structure in the *Sorex vagrans* ovaries at hand. The fact that we were unable to find these structures in immature ovaries threw doubt on the theory that they were actually medullary cords. Hence we have used the non-committal term "testis" cords or tubules.

MATERIAL

The ovaries of twenty-four *Sorex vagrans* were studied. Nineteen of the animals were pregnant, two animals were mature and non-pregnant, and three were immature. Fixation of almost all of the specimens was carried out in the field in an alcohol-formaldehyde-acetic acid mixture. Stains used were either hematoxylin-eosin or Groat's four-color stain.²

We are indebted for the collection and preservation of this material to Dr. Walter W. Dalquest of the California Museum of Vertebrate Zoology and to Dr. Phillip L. Wright of the University of Montana. Dalquest's collection was made in the vicinity of Seattle, Washington; Wright's near Missoula, Montana.

¹ This work was supported by grants from the Wisconsin Alumni Research Foundation.

² Unpublished.

STRUCTURE

The "testis" cords first observed by us, and the great majority of those seen, are strikingly similar in appearance to embryonic seminiferous tubules. These represent their most mature form.

In structure, the mature "testis" cord has a consistent form which is altered only where connections are made to the cortex of the ovary or to the rete.

The mature cords seen in the medulla (figs. 1, 4, 5, and 11) have a well-defined tube-like shape with a definite basement membrane circumscribing the epithelium. There is no surrounding theca interna such as League and Hartman ('25) described around anovular follicles. The cells are peripherally arranged on the basement membrane without any uniform arrangement rather than a roughly radial orientation. Cell boundaries are indistinct. The nuclei are vesicular, round or oval, and show a prominent nucleolus. Mitoses are common. In the center of the tube is an area of undifferentiated, non-cellular material. This seems to be a syncytium composed of the inner cytoplasmic portions of the epithelial cells, and may in part be a product of the destruction of the constantly proliferating peripheral cells, since pycnotic nuclei are often observed in it. The cords do not ordinarily have a true lumen, but in the case of some of the largest tubules a partial clearing of the central zone can be seen as in figures 4 and 5. The cords are usually relatively short and seldom connect with one another. In those ovaries in which only a few cords are seen they may be nearly straight. On the other hand, in the ovaries in which the tubules are abundant, the shape is more apt to be knot-like (fig. 10), or hooked and tortuous (fig. 4). In either case, they end abruptly without much tapering at the ends. Branching of the tubules was not observed in the fully mature forms. No ova were ever found inside the tubes.

In the region of the rete where the cords are often attached to the rete tubules, the typical structure described above is altered. A gradual transition takes place to the form of tubule normally seen in the rete. The central undifferentiated zone disappears and a definite lumen can be observed. The nuclei of the testis tubule become smaller and flattened and lose their characteristic oval or round shape (figs. 5 and 11). The whole structure is much smaller and more irregular in shape. In the nineteen animals in which well developed "testis" cords were observed, three showed connections between the cords and the rete. However, because the connecting tubules are so small and tortuous, it was impossible to find any single section that demonstrated the complete

connection clearly. Figure 1 shows the best single example that could be found.

Where the cords reach to the cortex the basic structure is again altered. The basement membrane is lost on the side closest to the surface epithelium of the ovary and a column of the smaller, darker cortical cells can be seen apparently proliferating inward making connection with the tubule (figs. 6, 7 and 8).

OCCURRENCE

At first it was thought that these "testis" tubules were chance occurrences, or vestigial structures in the adult *Sorex vagrans* ovary. After all the ovaries had been examined it became evident that they were not present in the more immature individuals, and in certain adults. This suggested a definite relationship might be demonstrated between the cyclic development of "testis" tubules and the progression of the reproductive cycle, but the small number of *Sorex* ovaries available made it impossible to definitely establish any such relationship. Nevertheless, examination of table 1 does indicate that mature "testis" tubules are to be found neither in the immature nor in preimplantation stages of pregnancy. It also indicates development of the rudiments of these structures in late immaturity and during the preimplantation period, and that these become fully developed tubes during mid and late pregnancy. The fly in the ointment is, of course, the failure to find any trace of either rudimentary or mature tubes in certain adult and pregnant individuals such as nos. 18, 10, 9, and 17. It is possible that the second ovaries of 10 and 9 might have shown them had they been available for sectioning. Then too, nos. 18 and 17 were Montana specimens, and could conceivably differ in anatomical detail from the Washington variety. Also there is always the possibility of mistaken identity of species, but that is rather unlikely as both the men who collected these specimens are very competent mammalogists. The final explanation, other than an unexplained species variability, is a technician's confusion of records during the preparation of the slides, but we can find no evidence that this occurred.

Obviously, therefore, not much can be definitely stated about the occurrence of the "testis" cords in relation to the reproductive or life cycle, until a much larger series of animals is collected and studied. More careful records concerning each animal should then be kept. It is of great help in determining the approximate age and past reproductive history of a wild mammal to have data on such things as body length, size of nipples and presence or absence of matted hair around

TABLE 1

The occurrence and estimated relative numbers of "testis" cords in the ovaries of individual animals, together with other pertinent data. The individuals have been arranged in order with the earlier stages above and later ones below.

All the animals, except the two most immature ones (nos. 23 and 27), had some relatively large vesicular follicles, both atretic and apparently normal. Some of these appeared to be ripe in nos. 11, 25, 1, 6, 20, 8, 18, 2, 13, 10, 9, 4, 17, 7, 25, and 12. However, we have seen no undeniably ripe follicles in this species as we have no ovaries from animals that are known to be in estrum. The individuals of each of the following groups were in practically the same stage: —23 and 27, —23, 11, and 24, —5, 18, 2 and 13, —10, 9, 4, 17 and 7, —19 and 15. Attention should be called to the fact that there is considerable variation in the number and even in the presence of "testis" cords in some of these groups.

+ = present but scarce. ++ = average. +++ = numerous. ++++ = very numerous. — = none.

STAGE OF CYCLE	OVARY	"TESTIS" CORDS		CORPORA LUTEA			TIME OF YEAR		ANIMAL NO.
		Early	Mature	Early	Mature	Degenerate	Month	Day	
Immature	A	—	—	—	—	—	July	29	23
Immature	A	—	—	—	—	—	Apr.	17	27
Immature	A	++	—	—	—	—	Feb.	13	26
Tubal ova (1-2 cells)	A	++++	—	3	3	—	?	?	28
Unattached uterine blastocysts	A	+	—	—	5	—	?	?	11
	B	?	—	—	7	—			
Unattached uterine blastocysts	A	+	—	—	3	—	Apr.	8	24
	B	+	—	—	7	—			
Larger uterine blastocysts	A	+	—	—	12	—	Apr.	28	25
Neural groove	A	++	—	—	2 ±	—	Feb.	27	1
	B	+	+	—	5	—			
Gill slit	A	+++	+++	—	1	—	Apr.	7	6
	B	+++	—	—	4	—			
Late gill slit. 3 mm. (coiled)	A	++	++	—	4	—	Feb.	13	20
	B	++	++	—	2	—			
Very early limb-bud	A	++	—	—	3	2 ±	?	?	8
	B	+	+	—	3	4			
Mid limb-bud 6.5 mm. CR	A	+	+	—	3	—	March	17	5
Mid limb-bud 6 mm. CR	A	—	—	—	3	4	May	26	18
	B	—	—	—	3	6 ±			
Mid limb-bud 6.5 mm. CR	A	+++	++	—	4	—	March	10	2
Mid limb-bud 6.8 mm. CR	A	—	+++	—	3	—	Apr.	1	13
	B	—	+++	—	4	—			
Late limb-bud	A	—	—	—	3	—	Apr.	6	10
Late limb-bud	A	—	—	—	4	—	Apr.	1	9
Late limb-bud 7.5 mm. CR	A	—	++++	—	5	—	March	11	4
Late limb-bud 7 mm. CR	A	—	—	—	4	—	May	26	17
	B	—	—	—	1 ±	—			
Late limb-bud. 8.3 mm. CR	A	+++	+++	—	4	4	Apr.	28	7
Late fetus 10 mm. CR	A	++	+++	—	4	1	Apr.	16	19
	B	++	+++	—	3	6			
Near term fetus	A	+	—	—	3	—	?	?	15
	B	—	—	—	2	2			
Post-partum	A	++	—	—	6	—	Apr.	28	25
Post-partum and probable estrum	A	+	+	—	3	—	?	?	12
	B	+	++	—	3	—			

them, whether or not milk can be expressed from them, and the size and histology of the glands themselves.

ORIGIN AND DEVELOPMENT

In attempting to reconstruct the development of the cords already described, it is logical to reconsider their occurrence and structure first. Are these "testis" cords vestigial structures? The evidence suggests that they are not. Since their structure closely resembles that of primary sex cords, it seemed probable at first that they are simply remnants of the embryonic medullary cords. On the other hand, the fact that the cords were not found in any of the immature animals studied and were seen only in mature animals, strongly indicates that they are not vestigial structures. However, the absence of cords from some adults leaves open the possibility that all the immature specimens we examined were individuals destined to develop into adults without cords. The abundance of mitotic figures in the cords shows that they are probably rapidly developing units and are not merely maintaining themselves.

It is impossible to state with certainty whether they are truly cyclic in their origin because the number of animals studied was so small. However, the fact that they were found only in pregnant or parous animals suggests that they have a relationship to the reproductive cycle. The further fact that all stages in their development are seen within a relatively short period of the cycle, coupled with the observation that mitotic figures are numerous, indicates that the cords develop rapidly in response of some definite stimulus received during the reproductive cycle.

The structure of the cords offers several additional clues to their probable development and origin. Although they are sometimes connected to both the rete and cortex, the character and arrangement of cells, the lack of a lumen except in the region of the rete, and the presence of an undifferentiated central zone all suggest that they are in some way analogous to the primary sex cords and arise from the cortex. Further evidence of this cortical origin was found upon examination of the surface of the ovary. In some of the ovaries the mesothelial covering had disappeared in spots; and the cortex, to a considerable degree, had taken on a completely uniform appearance. Epithelial cells could no longer be distinguished from the underlying cortical cells. This condition has been described by Gruenwald ('42) in his discussion of primary sex cord development. In these adult animals this process does not involve the entire cortex, but only parts of it. In the

areas that have reverted to this condition, an increased proliferation of cells can be noted. This becomes expressed in the formation of cords or columns of cortical cells which are pushed down into the underlying medulla. These columns of cells are the early stages of the cords. Figures 2 and 3 show early cortical cords extending down into the medulla. Figures 6, 7 and 8 show the absence of an epithelial covering on the cortex and two well formed cords still connected to the surface. After the invasion of the medulla by this cortical tissue, the majority of the cords become broken up into nests of cells which are isolated from each other. These cells become changed in appearance and lose their cortical characteristics. They become larger, lighter staining, more oval, and have a more vesicular nucleus. Figure 3 shows nests of cells which have acquired some of the characteristics of "testis" cords, but have not as yet been organized into the definitive structure.

The final step in the process occurs when the unorganized nests of cells assume the tube-like shape and acquire a basement membrane upon which they arrange themselves. Figure 10 is of a group of cells near completion of this process. At this stage irregular shapes are quite common until a basement membrane completely circumscribes the cord. As the cords become more mature the central syncytial area becomes more pronounced. The most mature cords show a partial dissolution of this area with lumen formation as in figures 4 and 5. In some ovaries it was possible to trace unorganized clumps of cells into well-formed "testis" tubules.

Upon consideration of all the facts which can be ascertained from the occurrence, mature structure, and development of the "testis" cords of these ovaries, it appears probable that these structures are not vestigial, that they have some cyclic relationship to reproduction, and are structurally similar to primary sex cords and immature testis tubules. Their early development parallels the development of primary sex cords. Are they then really homologous to seminiferous tubules? We believe that they are. The difference in development lies mainly in the fact that the cortex, although reverting to a primitive condition in part preparatory to the formation of the cords, retains its mature function in that it simultaneously is continuing the production of ova and primordial follicles (fig. 3). This observation was repeatedly made in the examination of those cortices which were actively forming cords.

The final conclusion that one must draw after consideration of the above observations is that the cells of the cortex are the ancestors of the "testis" cord epithelium of these ovaries, and that these cortical cells are pluripotential in nature since they are capable of partial

regression to a state resembling embryonic mesenchyme while other cells retain their mature functions in follicle production.

GENERAL DISCUSSION

We have not attempted to review the literature in ovotestes in mammals, as it seems to be entirely concerned with the relatively rare instances of true hermaphroditism. Nielson ('41) described such a case in the pig, and it may be found interesting to compare his figure 4 with our figures, as there is considerable similarity in the appearance of the tubules.

So far as we can discover there is no literature definitely dealing with structures of the sort we have found in *Sorex*. However, there are a number of papers on "anovular follicles", some of which describe structures which may be comparable. Regaud and Lacassagne ('13) described anovular follicles in the cortex of the rabbit ovary which bore some resemblance to what we consider to be younger stages of the "testis" cords of the shrew. League and Hartman ('25) described certain structures in opossum, monkey, and armadillo ovaries which they called anovular follicles. It is our opinion that some, not all, of these may have been "testes" cords (see their figures 9, 10, 11, 14, 15) with collections of interstitial tissue, not theca, around them. At least there is a striking resemblance between the cords which we saw and some of the "anovular follicles" depicted in their paper.

More or less incidental mention of both anovular follicles and medullary cords has occurred many times in the literature on the ovary, but no one has apparently seen ovaries in which they reach anywhere near the great development seen in *Sorex vagrans*. Brambell ('35) does not mention them in *Sorex araneus*, nor does Pearson ('44) in *Blarina brevicauda*. The senior author did not notice them in Sansom's series of *Crocidura* ovaries which he had the privilege of examining in Prof. J. P. Hill's laboratory at University College. Nor have we seen them in the few ovaries of other species of *Sorex* in our collection. It is probable, therefore, that they are not a common characteristic of the ovaries of *Soricidae*. Yet the fact that we did find them in seventeen out of twenty-one adult *S. vagrans* ovaries, and that they were very large or "mature" in eleven of these, indicates that they are something more than just an anomaly in this species.

SUMMARY

1. Cell cords closely resembling prepubertal testis tubules are to be found in most, but not all, ovaries of parous and of postimplantation stages of pregnancy of *Sorex vagrans*.

2. They are absent in the youngest animals trapped, but very early rudiments of them can be found in the cortical portions of the ovaries of late prepubertal animals and of animals in preimplantation stages of pregnancy.

3. These structures usually consist of short isolated segments of cords or tubes, but may be long and tortuous.

4. The deep ends of mature tubules are sometimes continuous with the rete tubules.

5. Immature or rudimentary tubules resemble cortical cords of the embryonic ovary, often being continuous with the surface epithelial layers. Rarely fully developed cords may be traced to these surface epithelioid masses.

6. In the relatively limited cortical areas where the cords are differentiating, or are connected, there is no distinguishable difference between the cells on the ovarian surface and those making up the bulk of the cortex in these regions. The whole mass seems to be continuous at its periphery with the distinct epithelium of the surrounding area.

7. Since these cords seem to arise from the cortical epithelium in a manner similar to the medullary cords of the embryonic ovary and testis, and since they attain a structure similar to prepubertal testis tubules, we believe they are homologous to these embryonic cords, although they are not derived from them, and to the tubules of the testes.

8. Similar but less prominent structures have occasionally been mentioned in the literature and have been seen in ovaries of several other species by the authors. They have sometimes been described as "anovular follicles".

9. There is no evidence to explain their function or reason for being.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

(All figures are of *Sorex vagrans* ovaries)

1 Obliquely longitudinal section of an ovary with its mesovarium and a segment of the glandular oviduct. "Testis" cords are abundant in the medulla and portions of the cortex. The epoophoron and rete show. The region of joining of the "testis" cords to the rete is near the center of the lower border of the ovary. The veins and lymphatics of the ovary are markedly dilated. This animal contained embryos of 7.5 mm. CR. in the late limb-bud stage. ♀ 4, B, slide 2, row 1, section 11. $\times 60$.

2 Groups of cells which are early stages in the formation of the "testis" cords can be seen in the cortex and medulla of this ovary. This animal contained embryos in the gill slit stage. ♀ 6, A, 2, 1, 6. $\times 70$.

3 Higher magnification of the cortex and adjacent medulla of the upper right portion of the ovary shown in figure 2. The upper margin of this figure is the ovarian surface. The print was trimmed very close to the surface, but not through it, although it appears somewhat that way because the adjacent ovarian capsule space is packed with red blood cells. Note the lack of an organized surface epithelium in these proliferative areas. The epithelium of the embryonic "testis" cords is being enveloped in a connective tissue capsule, but there is no basement membrane yet. $\times 320$.

4 Another section of the same ovary shown in figures 2 and 3, showing the region of the hilum where the mature "testis" cords can be traced to their connection with the rete. The typical coiling of the mature "testis" cords is demonstrated here as is also the fact that they occasionally have a lumen. ♀ 6, A, 2, 3, 4. $\times 70$.

5 Higher magnification of the mature "testis" cords seen in figure 4, showing their thin capsular connective tissue, basement membrane, and lumen with cellular debris containing both normal and pyknotic nuclei. The cord tapers toward the lower right corner to connect with the rete tubes. $\times 330$.

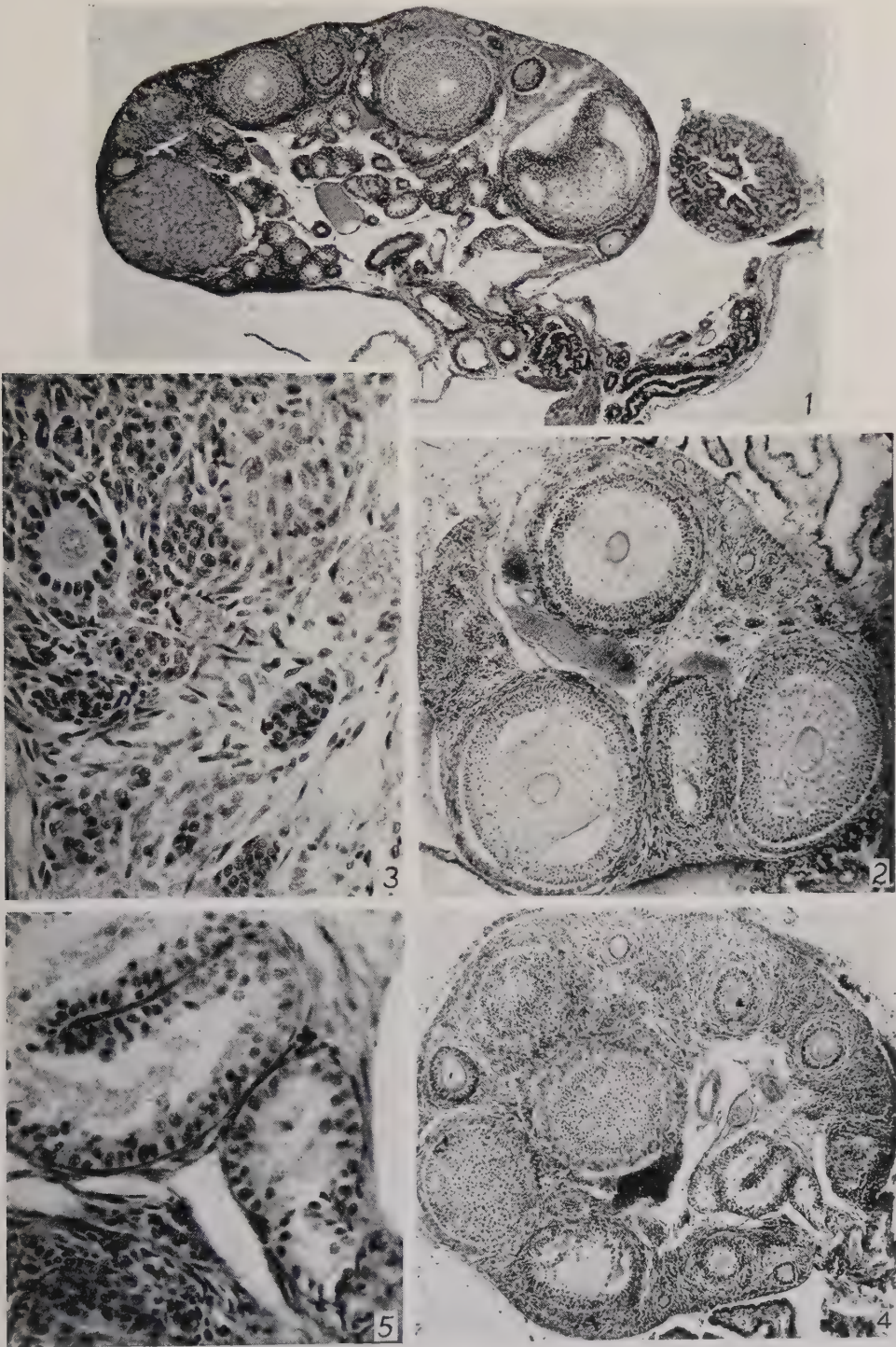


PLATE 2

EXPLANATION OF FIGURES

(All figures are of *Sorex vagrans* ovaries)

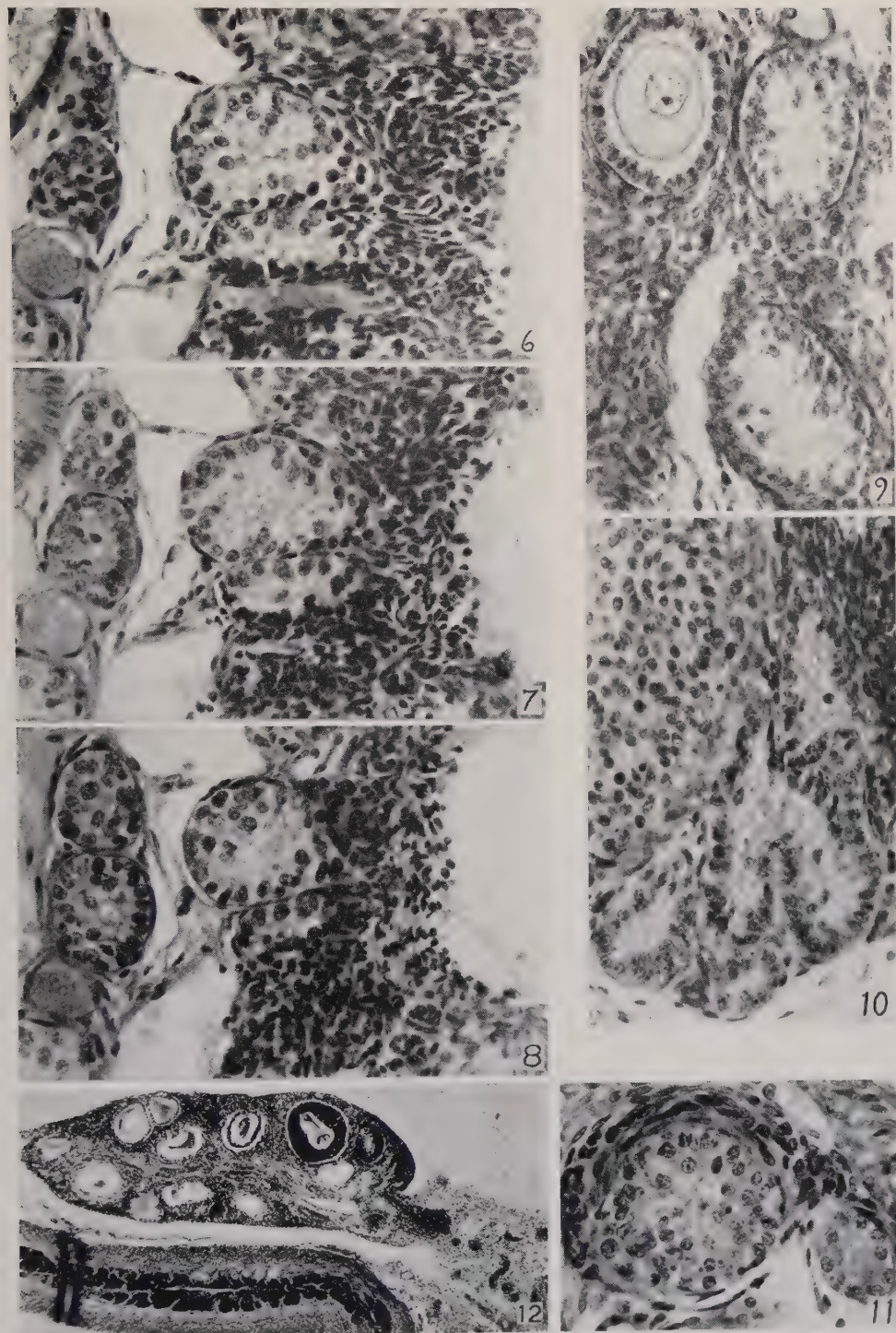
6, 7 and 8 These are consecutive sections showing two "testis" cords connected to the epithelioid cortex of the ovarian surface. There is no organized surface epithelium in such areas (cf. figure 3), and the cord epithelium is directly continuous with the epithelioid mass. This mass also grades imperceptibly into the ovarian stroma on its deep side, yet peripherally it is continuous with the typical ovarian epithelium of the surrounding relatively inactive area. This is in line with the mesenchymal origin of both the stroma and epithelium of the gonads as discussed by Gruenwald ('42). This animal contained embryos of 6.8 mm. CR in mid limb-bud stages. ♀ 13, B, 2, 1, sections 9, 10 and 11. $\times 325$.

9 These are mature "testis" cords in the cortex for comparison with a primary follicle as to size and type of epithelium. It is such cords as these that have been called anovular follicles. Note the mitotic figure in the lower cord. These are from the same animal shown in figure 1. ♀ 4, B, 1, 4, 9. $\times 325$.

10 A cord mass near the cortico-medullary border showing the branching of the apparent early cord masses as they begin to mature. The formation of the basement membrane is beginning and the nuclei are taking up their peripheral position. A portion of the granulosa of a medium large follicle occupies the upper left portion of the figure. This animal contained embryos of 6.5 mm. CR in the mid limb-bud stage. ♀ 2, 1, 3, 27. $\times 325$.

11 Sections of mature "testis" cords as they narrow to join the rete tubes. The transition in size to the narrow rete tubes (not shown) is gradual but the "testis" cord epithelium joins that the rete with considerable abruptness, an indication that although these tubes make connection with the rete they are probably not actually derived from it. The connecting cords and their cells are much smaller and more irregular than the typical "testis" cords, and their epithelial nuclei also lose their typical shape and structure (see also fig. 5). This is from the same animal as figures 6, 7, and 8. ♀ 13, A, 2, 5, 6. $\times 325$.

12 Immature ovary, mesovarium, and oviduct showing epoophoron cords and rete epithelial mass at junction of ovary and mesovarium. No medullary cords are found at these ages, indicating that the "testis" cords of the adult ovaries are not remnants of embryonic medullary cords. The absence of cords from some adults, of course, leaves open the possibility that all the immature specimens we saw were destined to develop into adult individuals without cords. ♀ 23, A, 1, 4, 2. $\times 75$.



HOW MUCH MODIFICATION OF THE BNA IS DESIRABLE?

In an earlier note this group expressed its conviction that the BNA system of anatomical terminology ought to be maintained in its original form until such time as modifications can be officially adopted by representatives of the several anatomical associations. It is our opinion that, pending ratification by such properly constituted authority, the use of substitute terms in textbooks and papers is detrimental to the best interests of science and should be actively discouraged. That there is a real need for improvement in the BNA is indicated by the number of revisions that have been proposed by various groups; but, in attempting any revision whatever, there should be kept in mind the disadvantages inherent in the modification of terms that have been in widespread use for the past half century and are found in practically all of the anatomical and much of the surgical literature of that period. Any far-reaching changes at the present time might impose a burden on students in the future who may wish to consult this literature, and might also affect unfavorably the attitude of those colleagues in other fields who only reluctantly adopt some of the terms which anatomists choose to endorse. It should also be recalled that the original formulation of the BNA was the result of many years of careful study by highly-trained anatomists, and that most of the considerations which influenced them still have weight.

But, while the reasons for proceeding with caution are many, it must be recognized that a kind of terminological crisis has arisen and that now is a propitious time to review the whole question of anatomical names with a view to determining what the real defects in the current system are, and what improvements may justifiably be proposed. In doing so, it may be well to bear in mind some of the tenets of the original commission, considering them in the light of past experience and current outlook. Official action taken by any regularly-constituted group will no doubt be on the conservative side; but it may be profitable for participants in preliminary and informal discussions to allow themselves considerable freedom in expressing their ideas. In that way all aspects of the question will be generally understood to the end that, when a revised system finally is accepted, it too may be expected to

remain relatively stable for at least another half century. Comments in this and subsequent notes are presented from this point of view.

Rigid standardization of terms may carry with it certain dangers that should be borne in mind. If any terms prove inaccurate or inapt, they may do much to discredit the whole system. In early stages in the development of a science, a certain amount of fluidity in terminology has its advantages; but in later stages, as concepts become clarified, some degree of nomenclatorial fixity becomes equally desirable. Gross anatomy has reached a stage where considerable crystallization of terminology is both feasible and desirable, as has already been demonstrated by the experience with the BNA. In standardizing the terms, however, there are several subsidiary questions that deserve consideration. One of the first of these concerns the relation to terminologies and usages in histology, embryology, and comparative anatomy, all of which are still in stages where considerable fluidity is desirable. But if any changes are to be proposed for terms which are also used in other branches of biology, some policy in regard to them should be determined in advance. There are obvious objections to the use of different sets of synonyms in comparative anatomy, embryology, and gross anatomy; and there are also very real difficulties in the way of finding a terminology that would be satisfactory to all three. This question, on which there is marked diversity of opinion, will be discussed in other communications.

In the choice of words not affected by the above considerations, a question arises as to the policy to be adopted in regard to the use of general terms. Shall we aim at a limited or a liberal number of terms to designate attributes which vary over a considerable range? It is obvious that no single term can adequately designate two different structures or relations; and the question as to how far somewhat different structures should be grouped under a single head becomes a matter of individual judgment unless some, probably arbitrary, rules are established. The question often hinges on just when a generic term becomes too inclusive to be really useful. For example, in the case of elevations and protuberances, terms could be used with almost any degree of inclusiveness, the limits being one term to include them all or a single term for each. Should we retain *trochanter* for one or two special structures when *tuberosity* might serve to designate them and other elevations as well? Our group is divided on this point, the majority preferring that only a minimal number of terms be frozen in any nomenclatorial system. It is argued that it should not be the purpose of such a system to take over the functions of descriptive or interpretative anatomy, but merely to provide an adequate set of basic terms whose correct applications will be clearly recognized.

Assuming that our terminology (whether it be called BNA or something else) will remain Latin in form, should we endeavor to establish a few definite rules for the combination and use of terms, even if doing so would involve minor changes in a considerable number of words? It is our opinion that we should; that the rules ought to be adopted first, and the necessary changes then made automatic. To illustrate the kind of change such a procedure might involve: the BNA at present has an *os ethmoidale*, so named because of its structure, and on the inferior nasal concha a *processus ethmoidalis*, so named because of its position. We would suggest such standardization in usage of adjectives that, under such circumstances, the bone would, as a matter of course, be called *ethmoideum* and the process *ethmoidalis*; or the adoption of some other convention which would clearly differentiate between the two meanings. While such rules might have to be arbitrary to a degree, it is believed that they could be made simple and that they would add greatly to the clarity of the terms to which they would apply without necessarily changing existing forms sufficiently to introduce any serious initial confusion. It would call for no change at all in the great majority of present BNA terms.

An important advance made on the adoption of the BNA was the omission of personal names except for the small number retained in brackets. It is our opinion that the retention of these names in brackets has served no useful purpose and has probably been harmful on the whole. Professor His, commenting on the matter some 50 years ago, thought at the time that good reasons could be adduced for retaining these names. He expressed the opinion that the ligaments of Poupert, Gimbernat, and Colles are easily remembered by students, who are likely to be led to learn more about the men whose names are thus signalized, while there is nothing, he thought, in any way stimulating in the terms *inguinale*, *lacunare*, and *reflexum*. It is believed that 50 years have shown the essential fallacy of Professor His' views on this point, that instructors in gross anatomy find the simple Latin terms more useful and that students find them easier to learn and more significant than the (usually mispronounced) personal names. If they had not been included in brackets in the first place, they would probably have long since ceased to be used even infrequently as synonyms in the anatomical laboratory. The experience with these names — neither officially retained nor clearly discarded — may point a moral for dealing with other terms which it may be desired ultimately to eliminate.

The chief purpose of this note is to suggest that before any detailed changes in terminology are proposed, the scope and basic principles of the system to be recommended be clearly defined in as few general

rules as may be compatible with soundness and clarity. Such a procedure would for the most part be simply a reaffirmation or modest extension of the BNA, and could probably be accomplished with considerable improvement and a minimum of disturbance of the present system.

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SIRENS, APROSOPI AND INTESTINAL ABNORMALITIES IN THE HOUSE MOUSE

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THREE FIGURES

Among mammalian monsters described in the literature one can distinguish roughly two groups from the developmental point of view: those which occur prevailing within certain specified genotypes and those which arise sporadically without reference to specific genotypes. The genetically determined monsters can be traced to gene-dependent and regularly appearing aberrations of development; while the sporadic type is caused by different accidental changes or traumata during embryogeny. The type of developmental aberration and the resulting malformation may be the same in sporadic and in genetically determined monsters; in fact, many of the genetic malformations have closely similar counterparts in the sporadic group. The differences between the groups lie in the mode of appearance of the malformations, which is regular in the genetic and irregular in the sporadic type; and in the mechanism responsible for the first aberrant step in embryogeny. This mechanism is probably the same in each monster of a particular genotype and phenotype, while it may be the same or different in sporadic monsters of similar phenotypes. Among genetically determined malformations in mammals we find: short-spine calves (Mohr and Wriedt, '30), tailless and kidneyless mice (cf. Dunn, '41), short-tailed rats with abnormal urogenital systems (Ratcliffe and King, '41), short-legged (Ancon) sheep and chondrodystrophic cattle (Crew, '23, Mohr, '29) — to mention only a few.

Sporadically occurring monsters of different types have been described in the zoological and veterinary literature but it is in the human pathological literature where they are found mainly; sireniform monsters in fact have up to now never been described in any mammal except man. In the latter case, however, it must be borne in mind that in the absence of sufficient family data the classification of a human monster

¹This research was carried out with the aid of grants from the Carnegie Corporation and the Fund for Research of Columbia University.

among the group of sporadic cases may not always be justified. Even with sufficient data it is not possible to draw a sharp line between the two groups of monsters, for there are cases where a large number of "sporadically" appearing monsters are found in one inbred strain of mammals and none in others. Such a case for example has been described by Dunn et al. ('42) who found that the appearance of tailless rats within certain families could not be accounted for by the action of particular genes nor could it be termed "sporadic"; it was due rather to the interaction of a genetic constitution favorable to taillessness with accidental developmental irregularities. A similar case is that of otocephaly in the guinea pig (Wright and Wagner, '34) and a comparable situation exists for the groups of monsters to be described in this paper.

The severe abnormalities reported here were noted in newborn mice in the course of studies on the developmental genetics of different genotypes. They appeared among the offspring of mice carrying different mutations at or near the *T* locus, the main effect of which is on the tail. A review of the literature on these mutations has been given by Dunn ('41).

The abnormalities described below had never been observed in any of the other numerous strains in this laboratory. While no single genotype can be made responsible for these malformations, their comparatively frequent occurrence in the mutant strains seems to be related to the presence in these mice of the mutations at or near the *T* locus.

Among these various newborn monsters three groups stand out with particularly striking abnormalities: the first group shows extreme malformations of the posterior region of the trunk and the extremities (sirenoid malformations); the second group comprises animals with striking abnormalities of the head while the trunk, extremities and tail appear perfectly normal (aprosopi); the third group comprises animals with severe intestinal abnormalities, i.e. complete absence of large parts of the intestine.

SIRENS

Five sirens were observed in the course of the dissection studies. They arose in five different matings and belong to at least three and possibly to five different genotypes. The first siren (fig. 1) was found stillborn in a litter from a mating of a heterozygous short-tailed female (*T*/+) by a homozygous fused male (*Fu*/*Fu*). Its genetic constitution was probably *Fu* +/+*T* but it might also have been heterozygous for *Fu* only (*Fu* +/+ +). Head, forelimbs and the entire anterior half of the monster appeared normal; the posterior half was considerably shortened and instead of two hindlegs there was one median leg. The



Fig. 1 Ventral view of first siren (no. 3008). Monopodal symmelic monster. Note absence of tail, anus and genital papilla.

skeleton of this symmelic monster as studied in the cleared specimen stained with Alizarin Red S showed several abnormalities. The head and anterior extremities were normal. The ribs were normal except for a few slight fusions. The sternum was either missing entirely or extremely reduced. Only cervical and thoracic vertebrae were present in the spine and even these were rudimentary only; lumbar, sacral and caudal vertebrae were missing entirely. The single median limb consisted of one femur, two tibiae and one abnormal foot.

In addition, several abnormalities of the viscera were observed at dissection. There was a situs inversus of the heart. Lungs and liver were too necrotic for diagnosis. The lower part of the intestine was atretic; the entire colon and the rectum were missing, and the intestine ended blindly near the ileocaecal region. The adrenals appeared normal in their normal position. No viscera were found posterior to the adrenals: kidneys, ureters, bladder, urethra, gonads and genital ducts were missing entirely.

The second siren to be observed arose in a cross of two animals both of which were heterozygous for fused (Fu) and the recessive mutation t^o ($Fu +/+ t^o \times Fu +/+ t^o$). The monster was thus either $Fu +/+ t^o$ or $Fu +/Fu +$ since t^o/t^o is lethal in early embryonic stages and prevents recombination. The siren was stillborn and had two tiny stumps at its posterior end, rudiments of the two hindlimbs. Upon study of the cleared and stained skeleton (Alizarin Red) the following rudimentary hindlimb structures were found: two short femora, very close to each other inside the posterior trunk; remnants of the metatarsi and some faintly ossified structures which might have been either remnants of the tibiae and fibulae or of the tarsi. The pelvic girdle was missing entirely. The spine was present throughout the cervical and thoracic region only, but even there it was abnormal, all vertebrae being open dorsally. The ribs showed extensive fusions, and the sternum was abnormal, all stenebrae being fused with each other. Head and anterior extremities were normal.

The intestine was atretic again in its posterior part and ended blindly near the ileocaecal region. No kidneys, ureters, bladder or urethra were found; but there were two ovaries and uterine horns which however did not join.

The third siren (fig. 2) arose from a cross of two tailless animals of the tailless A line (T/t^o) both of which were heterozygous for "u", a recessive tail mutation affecting the urogenital system also (Dunn and Gluecksohn-Schoenheimer, '44). The genetic constitution of the monster was T/t^o and in addition it was probably homozygous for "u". Skeleton:

this animal was a bipodal symmelic siren, with one median hindlimb structure which upon closer examination appeared to contain all the elements of the two hindlimbs, although arranged abnormally. The elements of the pelvic girdle were all present, in abnormal arrangement also, the ischia being fused with each other. All vertebrae along the spine were completely abnormal, particularly in the lumbar and sacral region, all of them showing wide clefts dorsally. The ribs were fused

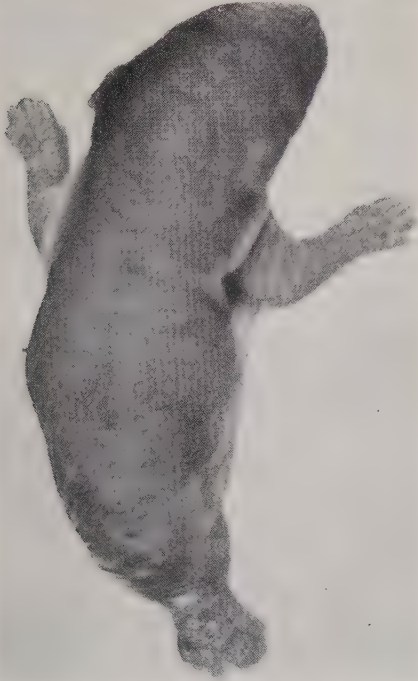


Figure 2

Fig. 2 Dorsal view of third siren (no. 1540). Bipodal symmelic monster. Tail, anus and genital papilla are missing.

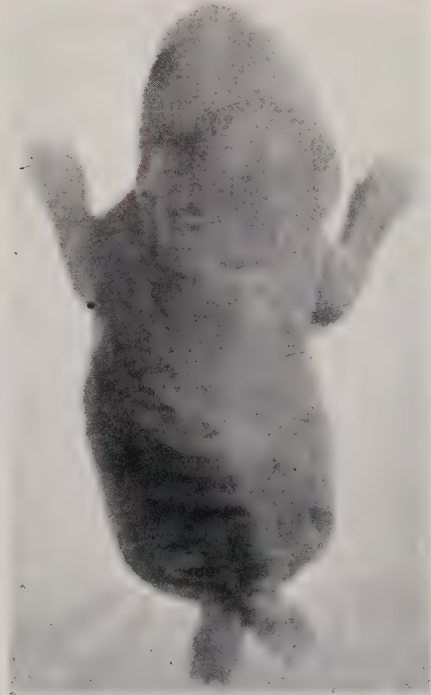


Figure 3

Fig. 3 Dorsal view of fourth siren (no. 3871). Bipodal symmelic monster, proximal parts of hind limbs fused inside trunk. Tail, anus, genital papilla missing.

partly and so were the stenebrae, thus giving the entire sternum an abnormal appearance. The viscera showed the same kind of abnormalities as described for the preceding cases: the intestine ended blindly near the ileocaecal region; kidneys, ureters, bladder and urethra were missing; ovaries and uterine horns were present but the uterine horns did not join. Adrenals and all anterior organs appeared normal.

The skeleton of the fourth siren (fig. 3) was not cleared and a detailed description is therefore not possible. The hind limbs were fused inside

the trunk, and the only structures appearing externally were the two hind feet which, although very closely approximated, were not fused. This monster was born alive but died soon after birth. It arose from a mating of a heterozygous normal tailed female ($+/t^o$) by a heterozygous short-tailed male ($T/+$). Both parents were heterozygous for "u" and the genetic constitution of the siren probably was $T/t^o u/u$. As in the three other cases described, there were no excretory openings. Viscera: the intestine again ended near the ileocaecal region with colon and rectum missing entirely. A median structure, representing fused hydrotic kidneys and ureters, was present, but bladder and urethra were missing. Ovaries and uterine horns were found, but the latter again failed to join. The adrenals appeared normal as did the rest of the anterior structures.

The fifth siren was found in a litter from a mating of two fused heterozygotes ($Fu/+ \times Fu/+$); its genetic constitution probably was Fu/Fu . It was stillborn. Externally it showed the following abnormalities. The hindlimbs were fused along the femora, but distal to the knee joints they were separate. The monster was tailless and genital papilla and anal openings were missing. Upon dissection the skeleton of the hindlegs, i.e. femora, tibiae, fibulae, tarsi and metatarsi, was found to be normal. But the pelvis was very abnormal resulting in the abnormal posture of the hindlegs; the ilia were present but unattached to the spine and there was a solid ossified mass to which ilia and femora attached and which probably represented the remnants of the ischia and pubes. The spine ended with two or three abnormal lumbar and all elements caudal to that were missing. The viscera of this siren showed the following abnormalities: the intestine (i.e. colon) ended blindly a short distance below the ileocaecal region. The urogenital organs were reduced to very small rudiments located in a lump of tissue in the lumbosacral region: two tiny vesicles probably represented remnants of the gonads (ovaries?) and several ducts were either ureters or uterine horns. Bladder and urethra were missing completely as were the kidneys. The remnants of gonads and ducts connected with a lump of soft tissue imbedded close to the posterior end of the spine (in the lumbar region). Because of its necrotic condition, this tissue could not be preserved and sectioned for diagnosis; but it contained spinal cord tissue, and thus this monster probably represented a case of *rhachischisis partialis* (*spina bifida*).

The features common to the sirens described above are thus: reduction or absence and fusion of the elements of the hindlimb skeleton, resulting in the least extreme cases in a fused median hindlimb contain-

ing the elements of both limbs; furthermore, reduction or absence of the pelvic girdle, severe abnormalities along the spine, fusion of ribs, but on the other hand, a perfectly normal head and forelimb skeleton. The viscera are abnormal in the posterior trunk region; the intestine is atretic from the ileocaecal region on and the urogenital system is highly reduced. The gonads and genital ducts are affected to different extents.

The adrenals were normal in all cases and thus mark a sharp boundary. Posterior to the adrenals every organ may be malformed but anterior to the boundary hardly any abnormalities were observed. The only abnormality of the anterior organs noted was a situs inversus of the heart.

Of the five sirens three were females; another one was probably a female (the nature of the gonad could not be ascertained without doubt) and in the fifth siren the gonads were missing entirely. Due to the small number of cases, however, no significance can be attached to the distribution of sex among the sirens.

Sporadic cases of human sirens with malformations such as the ones reported here have been described frequently (for extensive literature cf. Kampmeier, '27; Feller and Sternberg, '31, and Gruber, '37). The anatomical details are quite similar and include more or less complete fusion of hind limbs, pelvic abnormalities, absence of the posterior part of the spine, anomalies of the remaining vertebrae and abnormalities of the urogenital system. Both Gruber and Kampmeier mention in their reviews of sirenoid human monsters the absence of a higher frequency of these monsters among siblings and stress the sporadic nature of this type of monster. Kampmeier emphasized the fact that not a single case of siren formation in other mammals was to be found in the literature. In both these points our material differs from that reported by Kampmeier and Gruber. Also, a predominance of the male sex among sirenoid monsters was reported by Kampmeier in contrast to our material.

Feller and Sternberg in their excellent series of papers (cf. '31, '38) thoroughly describe and discuss human sireniform monsters. According to their classification all monsters with median symmetrical defects of the posterior trunk region should be included in the group of sirens. Without attempting to dispute the justification of their classification we have included in our report only those cases which would be termed "sirens" according to the popular concept, i.e. animals with fused and more or less perfectly developed posterior extremities like those mythical beings supposedly representing hybrids of man and fish which gave the name to the whole group. Among the human monsters described by Feller and Sternberg there are several cases closely resemb-

ling in their anatomical details the types of sirens reported here, while the cases of abnormalities cited by them from the veterinary literature would not be called sirens according to our nomenclature.

APROSOPI

The six newborns with different degrees of head malformations belong to two or three genetically different groups in regard to the mutations at or near the *T* locus: two of them show tail abnormalities in addition to the head malformations while the remaining four have normal tails. The malformed newborns cannot be classified strictly as aprosopi since their abnormalities vary in degree. By using the term "aprosopus" we wish to indicate only that the abnormalities observed and described consist in severe reduction or complete absence of certain head structures.

The first monster arose from a cross of a normal-tailed female which was heterozygous for the two recessive tail mutations t^0 and t^1 to a short-tailed male carrying the two dominant tail mutations Fused *Fu* and Brachy *T*, i.e. $t^0/t^1 \times Fu +/+ T$. The lower jaw was missing in this animal and the mouth slit was absent; in addition to the head and tail malformations, the left kidney, ureter and bladder were absent, while the urethra was normal and its course could be followed into the genital papilla. The gonads and genital ducts as well as the intestine were perfectly normal.

The second monster in this group was found stillborn in a litter from a mating of a female $Fu +/+ t^0$ by a short-tailed male $T/+$. It was a tailless female and its genetic constitution was either $T +/+ Fu$ or T/t^0 . The head was highly abnormal: it was reduced in size and abnormally shaped; eyes and mouth were missing entirely. A protuberance on the top of the head might have been a remnant of some more anterior structures and a conical proboscis-like process on its ventral side the rudiment of nose and snout. The left ear seemed normal while the right ear was shifted ventrad. Intestine and urogenital system were normal.

The third monster was a normal-tailed male which appeared from a cross of a normal-tailed female heterozygous for the two recessive tail mutations t^0 and t^1 (t^0/t^1) to a tailless male which carried the two dominant tail mutations *Ki* and *T*, and was also heterozygous for either t^0 or t^1 . The genotype of this normal-tailed monster could only be t^0/t^1 ; and head abnormalities such as microcephaly and microphthalmia have been found occasionally in animals of this genotype (Dunn and Gluecksohn-Schoenheimer, '43). The head of the animal was small and

pointed, the mouth slit was absent, the right eye was missing and both ears were dislocated ventrad. The following abnormalities of the viscera were found: the right ureter, instead of joining the bladder, joined the vas deferens; both testes were smaller than normal, a phenomenon frequently observed in t^0/t^1 males.

The fourth abnormal newborn in this series was a normal-tailed male from a cross of a normal-tailed female (t^0/t^1) to a tailless male ($Ki\ T/t^n$); its genetic constitution must have been t^0/t^1 , a genotype in which head abnormalities are found occasionally as mentioned above. This "microcephalos" had no eyes; both ears were shifted ventrad; the lower jaw was much reduced and the mouth slit was missing. Intestine and urinary system were normal; the genital system was too necrotic for diagnosis.

Both the fifth and the sixth monster arose from matings of tailless animals of the two different lines A (T/t^0) and 29 (T/t^1); both were normal-tailed females of the constitution t^0/t^1 . In one of them the head was much foreshortened, the eyes were missing entirely and the head rounded off in front of the ears which were shifted ventrad (grade 10, Wright and Wagner, '34). A small median ventral structure might have been a remnant of snout and nose. The viscera were normal. The head of the sixth monster was slightly longer; it was pointed and ended in a proboscis like structure. Both eyes were missing and the ears were shifted ventrad. The viscera were normal.

Of the six monsters with head abnormalities only one showed visceral malformations also. These were of a type not infrequently associated with the mutation fused (Fu) which the monster might have carried. The abnormalities in this group were heavily concentrated in the head and thus we have here the counterpart of the sireneid malformations described before where mainly the posterior trunk region was affected.

This group of monsters reminds us in its anatomical features of the otocephalic guinea pigs described by Wright and Wagner ('34). They classified the otocephalic monsters into seventeen grades of abnormality, ranging from "short lower jaw" through "single median ear" to "single median nostril" and "single median eye" to an aprosopus with a single median ear. All these abnormalities are assumed to trace to defects of a small number of centers which in turn may be related to inhibition of anterior medullary plate and ectodermal placodes and interference with formation and migration of neural crest cells and possibly even further, to inhibition of prechordal plate. Both hereditary and environmental factors cooperate in the production of these monsters as they do in the cases described here.

INTESTINAL GAPS

The third group of monsters comprises a number of animals with severe intestinal abnormalities. In these, considerable parts of the small intestine were missing. Atresia of a large part of the duodenum and of the entire jejunum and ileum was found in each case. In the eleven cases observed the stomach seemed normal; a short piece only of the duodenum was present; most of it as well as the entire jejunum and ileum were atretic. A large part of the colon was atretic also, but the descending colon, the rectum and the anus were perfectly normal. In a few cases the proximal and distal ends of the interrupted intestine formed blind sacs, in other cases the ends were open and a mass of mesenteries was found between them; in still others a string of connective tissue connected the two ends. Sometimes the duodenum was dilated in front of the beginning of the atresia. No study of the development of this abnormality has been made, and guesses as to its mode of formation can be based only on the morphological study of the malformation itself.

Since in a number of the cases the umbilicus was found to be open, it is conceivable that there existed an umbilical hernia with a protrusion of part of the intestine which remained unobserved because the mother ate it immediately after birth. Whether this hernia would have represented a perpetuation of the fetal condition around the middle of the gestation period when part of the small intestine protrudes into the umbilical cord or whether it would be due to a secondary protrusion of the viscera after primary withdrawal, cannot be decided now. In fact the hernia itself was never actually observed but only surmised.

However, the close correspondence of the parts missing in the cases of intestinal atresiae described here with the structures contained in the physiological umbilical hernia — namely the small intestine, caecum and part of the colon — points strongly to the failure of the herniated structures to be drawn into the body as an explanation for the lack of parts of the intestine. Two other points in favor of a secondary destruction due to the hernia rather than a primary inhibition of formation of the missing parts of the intestine are, first, the fact that the proximal and distal ends of the interrupted intestine were found to be open or forming blind sacs and secondly the presence between them of mesenteries and strings of connective tissue. If aplasia (i.e. failure of formation) rather than secondary destruction was responsible for the absence of intestinal structures in these newborn, one would expect the rest of the intestine to be continuous rather than interrupted in the manner described.

The monsters in this group arose from different genetic crosses. Five of them were from crosses of normal-tailed females (t^0/t^1) to tailless males ($Ki\ T/t^n$), three of them from crosses of two tailless animals of the different lines A (T/t^0) and 29 (T/t^1) and one from a cross of a normal-tailed female (t^0/t^1) by a tailless male (T/t^0). In all these cases, the genotype of the monsters must have been t^0/t^1 since they had normal tails.

There were two additional monsters with the same abnormalities which carried only one of the recessives ($+/t^0$ or $+/t^1$); and these arose from a cross of Brachy $T/+$ to t^0/t^1 and to $+/t^0$. Thus, the same situation prevails here which was mentioned in the two other groups of monsters: no particular single genotype can be made responsible for the formation of these abnormalities. Among the "intestinal gap" monsters we find two genotypes (t^0/t^1 and $+/t^0$ or $+/t^1$), with t^0/t^1 containing the bulk of the monsters (9 out of 11). Embryonic and post-natal head abnormalities, high prenatal and postnatal mortality and male sterility have been mentioned previously as attributes of the t^0/t^1 genotype (Dunn and Gluecksohn-Schoenheimer, '43). The occasional occurrence of severe intestinal atresiae may now be added to them.

DISCUSSION

We thus are dealing here with the appearance of many abnormalities in one genetic group. Similar genotypes, representing different combinations of tail mutations at or near the T locus (cf. Dunn, '41), are involved in all three of the groups of monsters described. None of these abnormalities can be ascribed to the specific action of any particular mutation or combination of mutations with the possible exception of the "intestinal gap" which was never observed in any but the t^0/t^1 or $+/t^0$ or $+/t^1$ genotype. The appearance in similar genotypes of monsters with malformations as severe as those described, but totally different from each other, seems to indicate rather some general fundamental disturbance of early development which may find its expression in abnormalities of quite different organ systems.

In all except two cases out of the twenty-two monsters recorded, one parent at least carried a mutation t^0 or t^1 which is known to suppress crossing over in its immediate neighborhood, and is thus suspected of being a sectional change in a chromosome, such as inversion. Such sectional changes often cause abnormalities in synapsis leading to duplications and deficiencies of chromosome segments. It is possible that the frequent association of these abnormalities with t^0 or t^1 in the parent is due to such a cause.

Although no embryological study of the material reported here has been made, it is to be expected on the basis of previous embryological experience with other malformations in tail and posterior trunk region, that these abnormalities trace back to early embryonic stages. Primary disturbances of early embryonic processes rather than secondary destruction of originally normal anlagen must be responsible for the altered morphology of the sirenoid and otocephalic monsters. This same point of view has been put forward in regard to sireniform monsters by Feller and Sternberg (*loc. cit.*) and Sternberg ('31) who emphasize the inherent nature of the embryonic defects leading to the abnormal development of the affected organs in contrast to older authors who tried to trace the origin of the abnormalities to embryonic accidents and traumata. As regards the failure of the herniated intestine to be withdrawn and the resulting intestinal atresiae, the primary causes are obscure.

The strange phenomenon of the apparently mutual exclusiveness of anterior and posterior malformations in our material remains unexplained. The possibility exists that those monsters in which both anterior and posterior structures were severely affected did not survive until birth and thus remained unrecorded.

SUMMARY

Three groups of monsters are described which were observed among the offspring of mice carrying mutations at or near the *T*-locus.

The first group comprises sirenoid malformations.

The second group contains monsters with severe head abnormalities.

In the third group monsters with severe intestinal atresiae are described.

The significance of the appearance of these monsters in the mu'ant lines is discussed.

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THE EFFECT OF TERMINAL CONNECTIONS ON THE CALIBER OF NERVE FIBERS¹

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FOUR FIGURES

Nerve fibers of different sizes (diameters) are represented in the fiber population of peripheral nerve in definite frequencies. This frequency distribution, conveniently referred to as the "fiber spectrum" (viz., fiber size spectrum), varies from nerve to nerve, but is relatively constant for a given nerve under normal conditions. The physiological properties correlated with fiber size (e.g. conduction velocity, threshold, susceptibility to noxious agents, etc.) have been extensively studied (Erlanger and Gasser, '37) and the functional significance of an orderly distribution of fiber sizes has been indicated. Yet, the developmental aspects of the problem have not received commensurate attention. Work on nerve "growth" has predominantly been concerned with the elongation and the increase in numbers of axons, without much concern for their subsequent growth in width. Except for a few comparisons between younger and older animals, establishing an increase in average fiber size during postnatal development (Dunn, '12; Hursh, '39), there is no descriptive record of how the various nerve fibers come by their final sizes, and even less is known about the dynamics of the process. Aside from the fact that the giant neurons of Mauthner have been shown to retain excessive size when the embryonic brain part containing them is transplanted to abnormal locations (Detwiler, '40; Oppenheimer, '42; Piatt, '44), no experimental facts have become known that would bear on the ontogeny of nerve fiber size.

In contrast, some substantial advances have recently been made in the study of the recovery of fiber size in nerve regeneration. The distal regenerating part of an adult nerve fiber is of much the same size order as the young nerve fiber of the embryo, less than 1 micron in diameter; that is, it measures only a small fraction of the old part of the axon

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from which it grows out. In order for the axon to resume nearly normal dimensions over its full length, the regenerated portion (in somatic fibers) will have to widen up to a hundred times. During this phase of spectacular growth, the adult regenerating nerve makes the most suitable object for experimental analysis of the factors determining fiber size.

Diameters of regenerating nerve fibers have been recorded both histologically (Davenport, Chor and Dolkhart, '37; Weiss, '37; Gutmann and Sanders, '43) and oscillographically (Berry, Grundfest and Hinsey, '44; Weiss, Davis and Mylander, quoted in Weiss, '44 c, p. 417, footnote). At the beginning of regeneration, the young sprouts of fibers of all sizes are all about equally thin. They then gradually expand, according to most observers, from base to tip (see also Rexed and Swensson, '40). Since different fibers expand at different rates, their size range increases, until a new stationary spectrum has been reached. How closely the restored spectrum resembles the original preregenerative spectrum, depends on the smoothness of the regeneration process. Restoration is most nearly complete after mere crushing of the nerve, while it is more disturbed following transection and suture (Davenport, Chor and Dolkhart, '37; Guttmann and Sanders, '43) and still more defective after cross union of nerve stumps of markedly different fiber content (Nageotte and Guyon, '18; Young, '43).

On what factors then does the size that a given fiber is to attain depend, and how do they operate? The following variables suggest themselves for consideration: size and metabolic activity of the nerve cell body; diameter of the proximal axon stump; number of branches produced during regeneration; character and condition of the distal pathway; mode and appropriateness of peripheral reconnections; and functional use. An experimental exploration of the problem has barely begun. Holmes and Young ('42) indicated that spatial limitations may prevent regenerated fibers from recovering full size, and Weiss and Taylor ('44 b) have demonstrated that a constriction at any point along the course of a regenerating fiber limits the size which the segment of the fiber lying distal to that point can attain. This, in conjunction with the observation that axoplasm tends to pile up in front of constrictions (Weiss, '44 a, '44 b), has been construed as evidence that fiber size depends upon the rate at which axoplasm is supplied to the fiber from its central cell body. About the same time, however, it was discovered that the terminal tissues likewise affect the size of the nerve fibers connecting, or failing to connect, with them. Weiss and

Taylor ('44 a) noted that when a stream of regenerating nerve fibers is divided and one half is allowed to reconnect with the periphery, while the other half is blocked and forced to remain unconnected, the blocked fibers remain distinctly smaller than the unconnected ones. It was in order to substantiate and amplify this earlier observation, that the present experiments were undertaken.

The experiments were designed to compare fiber size recovery in pairs of regenerating nerves, in one of which the fibers were allowed to regain peripheral connections, while in the other they were blocked. Regeneration was initiated in both nerves at the same time and the same level by a single crush. One of the nerves was then severed distal to the crush and its proximal stump was sealed off so as to prevent its fibers from reaching peripheral terminations. This nerve will henceforth be referred to as "capped," the other one as "connected". After 4 or 12 months' regeneration time, the fibers of the capped and connected nerves, as well as those of the normal nerves of the opposite side for control, were measured and compared.

MATERIAL AND METHODS

All operations were done in white rats of between 200 and 250 gm. body weight. In ten experiments, the two major divisions of the sciatic nerve were used, with the peroneal and tibial nerves capped in alternation. In three cases, motor branches of the facial nerve were used, the buccal and the mandibular, the former being capped in two cases, the latter in the third. The sciatic nerve was crushed high in the thigh, the facial nerve near its emergence from the foramen. The crushing was done with a steel forceps over a stretch of from 2 to 3 mm. The principle of the operation and the approximate relation between crush, cap, and sampling levels are illustrated in figure 1 for the facial group (for the anatomical details, see Greene, '35).

The capping was done as follows. In one group, the proximal nerve stump was inserted into a 6-8 mm. segment of closely fitting carotid artery, the open end of which was then tied off with silk thread, as in the earlier experiments of Weiss and Taylor ('44 a). In a second group, the epineurium of the proximal stump was first stripped back from the cut for a distance of from 2 to 3 mm., then the denuded nerve portion was trimmed, and finally the epineurium was returned to its position, drawn over the cut surface, ligated, and coated with a solution of methylmethacrylate polymer in acetone. The resin hardened within a few seconds and formed a permanent and tightly sealing cap over the nerve stump.

After the specified regeneration period, the nerves were reexposed. The stumps of the capped nerves were stimulated electrically to detect any fibers that might have escaped through defective caps. Then the nerves were excised, fixed in osmic acid vapor, embedded in paraffin, and sectioned transversally. Series of cross sections were mounted

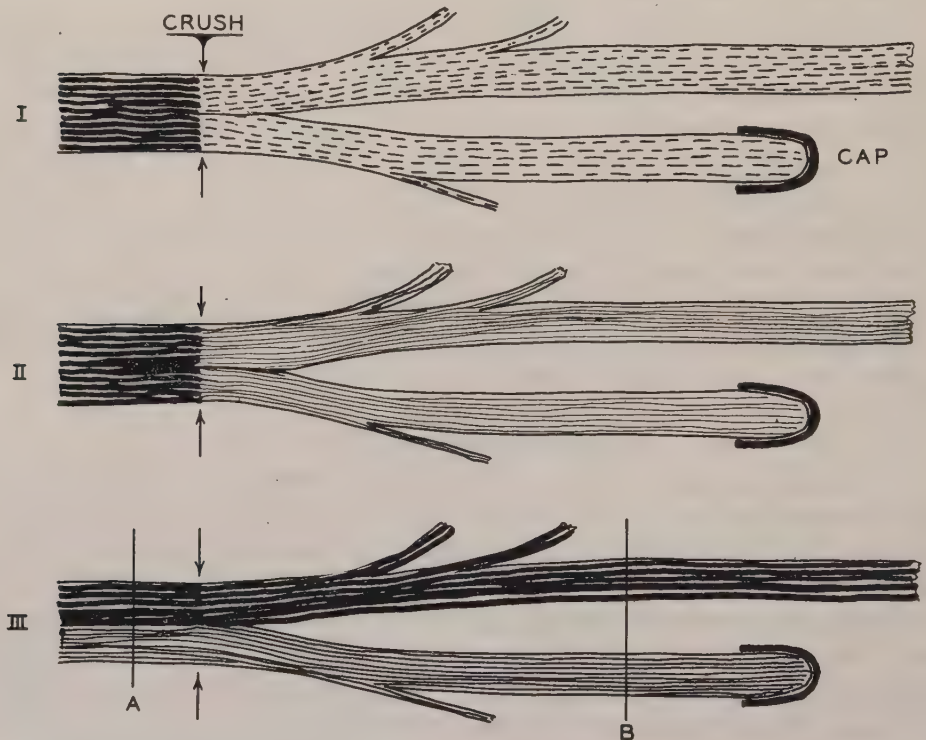


Fig. 1 Diagrams of the operation on the facial nerve and results. Common proximal stump (left) divides distal to crush level (arrows) into peripherally connected buccal (upper) and capped mandibular (lower) branches. I. Shortly after operation with distal stumps in degeneration. II. Early stages of regeneration. Young fibers in capped and connected nerves still of approximately equal diameters. III. Terminal stage of regeneration. Almost full recovery of fiber diameter in connected branch; undersized fibers in capped branch. Sampling levels A and B approximately 5 mm. proximal and 10 mm. distal to crush, respectively.

from sampling levels A (ca. 5 mm. proximal to the original crush; fig. 1, III) and B (ca. 10 mm. distal to the crush level). In the facial group, sections of the opposite control nerves were prepared from corresponding levels.

The fiber spectra were determined as follows. The nerve sections were projected through a microprojector with oil immersion lens on drawing paper at a magnification of ca. 920 times (1 mm. in the pro-

jection corresponding to approximately 1.1 micra). The whole field was subdivided into small parcels and the diameters of all medullated nerve fibers were marked down, going over one parcel after another. The diameter of each fiber cross section was traced in the direction most nearly representative of its true width. In fibers with oblong or irregular outlines, an estimate had to be made intermediate between the largest and smallest diameters. The recorded diameters were then measured and computed into equidistant size classes progressing in steps of 1 mm. (corresponding to 1.1 micra). Measurements were taken either by means of a celluloid wedge ruled at 10 mm. intervals with lines of 1, 2, 3, 4, 5, etc., mm. in length; or by a precision caliper, set first to correspond to the largest diameters and then narrowed in 1 mm. steps. With each setting, all diameters falling within that size class were checked off by crossing them with a pencil which, in turn, actuated an electric counter. This provision eliminated double counts as well as omissions.

From the total numbers of myelinated fibers in each size class, the percentage distribution according to size classes was computed, and these fiber spectra are given in graphic form below. In all graphs, blocks with double outlines represent spectra of normal control nerves, while black blocks represent the spectra of the regenerated nerves.

RESULTS

Figure 1 illustrates the sequence of events. Fibers of both nerves start to regenerate simultaneously from the crush level downward. Allowing for a week's delay at the lesion and a regeneration rate of about 3 mm. per day (Gutmann, Guttmann, Medawar and Young, '42), the majority of the fibers may be assumed to have spanned the 15 to 20 mm. between crush and the level of the cap within 2 weeks. Up to then, the conditions under which fibers in both nerves have proceeded have been similar. From this point on, they differ. The fibers in the capped nerve can proceed no farther, while those of the connected nerve continue to extend (fig. 1, II) until they reach the peripheral tissues. From previous experience (cf. Hines, Thomson and Lazere, '42), this may be estimated to require an additional 2 weeks. Consequently, in specimens sacrificed at 4 months or 1 year, the capped nerve had been devoid of peripheral contacts for the whole period, while most of the fibers of the connected nerve had been reconnected peripherally for 3 or 11 months, respectively. This difference in regenerative history has strikingly affected the fiber spectra (fig. 1, III): (1) The regenerated portions of the capped fibers have failed to expand to the dimen-

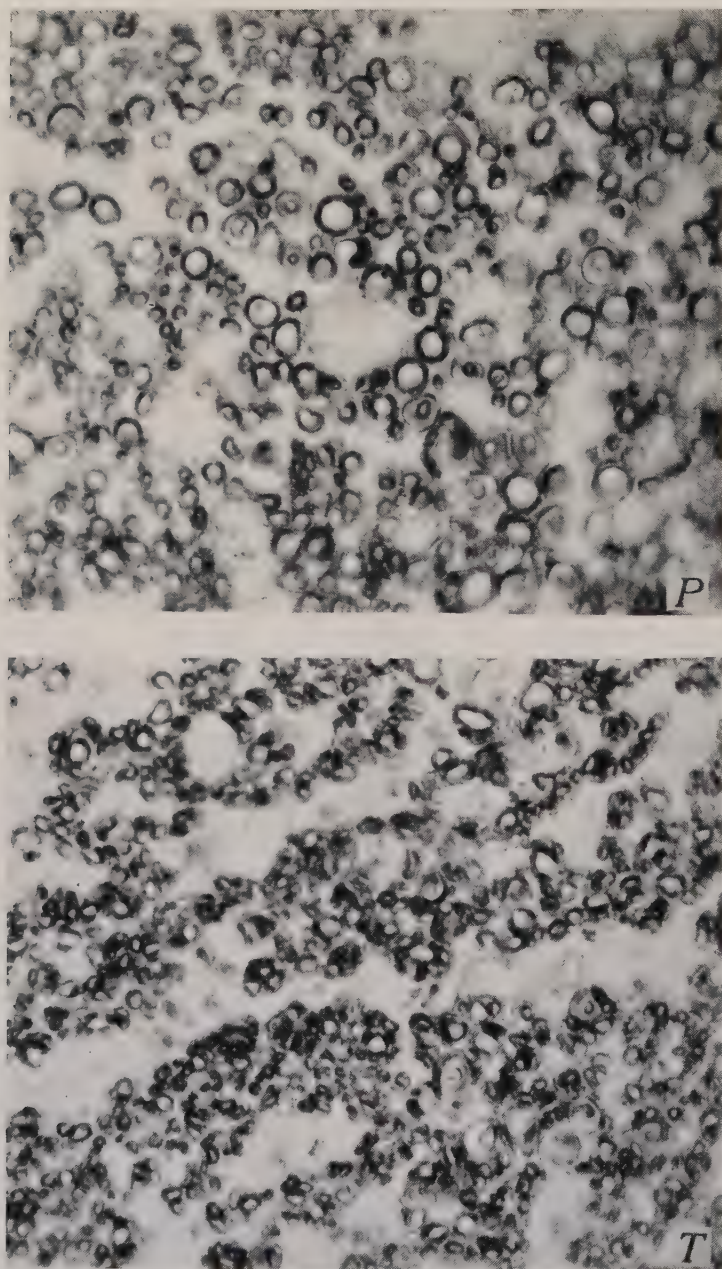


Fig. 2 Typical fields from the distal (regenerated) portion of a sciatic nerve with tibial branch (T) capped and peroneal branch (P) connected, showing pronounced difference in fiber size. Four months p. op. Osmic acid. $\times 625$.

sions of connected fibers. (2) The proximal old portions of the capped fibers have atrophied. A more detailed discussion follows.

The resin caps have effectively sealed the nerve stumps. They persisted as such and, in addition, provoked the formation of a fibrous foreign body capsule. When the regenerating fibers arrived at this end, they found no outlet and had to remain shut in, some presumably doubling back up into the nerve. The blind ends of fibers thus blocked have previously been described by Weiss and Taylor ('44 a).

Blocking of nerve regeneration by tied ends of artery proved less reliable, and in some cases a small amount of fibers escaped through the ligated end. However, the escaping fibers were so much delayed and so few in numbers that they did not affect the general results.

TABLE 1

Numbers and percentage distribution of myelinated fibers according to size in the peripherally connected peroneal branch and the capped tibial branch of a regenerated sciatic nerve, 4 months p. op.

NERVE		FIBER DIAMETER IN MICRA									TOTAL
		<1	1.1- 2.1	2.2- 3.2	3.3- 4.3	4.4- 5.4	5.5- 6.5	6.6- 7.6	7.7- 8.7	8.8- 9.8	
Peroneal (connected)	Number of fibers	47	780	989	531	273	160	81	17	2	2880
Tibial (capped)		774	7977	2831	888	335	119	27	4	..	12955
Peroneal (connected)	Percent- age of fibers	2	27	34	18	9	6	3	1	..	100
Tibial (capped)		6	61	22	7	3	1	..	..	..	100

Of the thirteen operated cases, one had to be discarded because of defective staining. In the remaining twelve cases a marked overall difference in fiber size between capped and connected nerves became immediately evident on microscopic inspection. Figure 2 shows typical samples of the distal (regenerated) portions (level B of fig. 1, III) of a sciatic nerve with capped tibial (T) and connected peroneal (P), 4 months after crushing. The fiber measurements of this case are listed in table 1.

The totals of regenerated branches can be compared with earlier counts of peroneal and tibial nerves. The normal peroneal of rats of the age class used contains below 2000 myelinated fibers (Tamaki, '33, '36). Regeneration after crushing yields a definite surplus of branches

(Greenman, '13). The present figure of 2880 distal fibers after 4 months compares with a previous count of 2350 fibers after 6 weeks under similar circumstances (Weiss and Taylor, '44 b). In contrast, the count of nearly 13,000 fibers in the capped tibial nerve is more than three times as high as that of a comparable unoccluded tibial regenerate (3647 fibers; Weiss and Taylor, '44 b). Blocking, therefore, has substantially raised the count of regenerated branches.

Even more remarkable is the effect of blocking on the fiber spectrum. A glance at figure 2 reveals a conspicuous size difference between the capped and the connected fibers, the former being much smaller on the average. This is borne out by the complete spectra listed in table 1. Of the two percentage figures for each nerve in each class, the higher one is given in bold face type. It becomes immediately obvious that the connected nerve greatly outweighs the capped nerve in the proportion of all larger fibers, while the capped nerve prevails in the two smallest fiber categories. Of the blocked tibial fibers, 67% measure below 2 micra, while 71% of the connected peroneal fibers lie above that value. This difference is even more impressive when one considers that of the two nerves the tibial normally contains the larger fibers (cf. table 2 of Weiss and Taylor, '44 b).

The eight remaining sciatic cases (three with capped tibial, five with capped peroneal) conform with the case just described. As sections of the connected and the capped nerves were mounted on the slides side by side, they could be viewed simultaneously and compared visually under the microscope. The result was of the kind illustrated in figure 2, namely, greater average fiber size in the connected nerve, regardless of whether it was the tibial or the peroneal. No quantitative determinations have been made of these cases.

Complete measurements have been taken of the regenerated segments in the three cases of the facial group (R309, R310, R311), preserved 1 year after the operations. The control nerves were likewise measured, with the exception of one mandibular nerve (R311); in its stead, the average of the two other mandibular controls, which were very similar, was used for comparison. Proximal measurements (level A) were taken in one case (R310), but it must be borne in mind that the nerve at this level contains fibers of branches not included at the farther distal sampling level B (cf. fig. 1, III). The results are listed in table 2.

These figures reveal the following facts.

(1) The normal complement of myelinated fibers is ca. 1500 for the buccal nerve, and ca. 1000 for the mandibular. Fifty to 60% of the fibers of both nerves lie in size classes 3 and 4, i.e. between 2.2 and 4.3

TABLE 2

Distribution of nerve fibers according to size in capped and connected branches of facial nerves, 1 year after crushing.

CASE	NERVE			NUMBERS (N) OF MYELINATED FIBERS OF DIAMETER (D) IN MICRA:										TOTAL	AREA (Σnd^2)
	Condition	Kind	Level *	<1	1.1-2.1	2.2-3.2	3.3-4.3	4.4-5.4	5.5-6.5	6.6-7.6	7.7-8.7	8.8-9.8			
R309	Control	Mand.	B	..	42	365	299	147	152	59	11	..	1075	20984	
	Control	Buc.	B	..	59	396	459	335	106	38	7	..	1400	26145	
	Connected	Mand.	B	38	338	578	519	422	172	72	12	..	2151	35929	
	Capped	Buc.	B	663	2284	819	171	16	3	..	..	..	3956	19427	
R310	Control	Buc.	B	1	254	441	442	241	137	37	6	..	1559	25211	
	Control	Mand.	B	3	106	240	299	226	129	23	11	1	1036	19607	
	Connected	Buc.	A	157	935	921	756	501	203	80	18	4	3575	49511	
	Connected	Buc.	B	272	1013	465	527	244	114	30	2	..	2668	28743	
	Capped	Mand.	A	204	1510	625	355	140	35	9	..	..	2878	22750	
	Capped	Mand.	B	421	1967	640	149	12	..	..	..	..	3189	16733	
R311	Control **	Mand.	B	1	74	303	299	186	140	41	11	..	1055	20207	
	Control	Buc.	B	2	155	358	368	366	152	38	3	..	1442	26406	
	Connected	Mand.	B	18	402	489	519	336	138	10	7	1	1950	30321	
	Capped	Buc.	B	312	1890	833	186	11	2	..	..	..	3234	18692	

* According to figure 1, III.

** Average of R309 and R310.

micra. Maximum fiber diameter is 9 micra, and the mandibular contains a significantly higher proportion of large fibers than does the buccal.

(2) Unblocked regeneration in connected nerves has produced nearly 2700 distal branches in the buccal (R310) and 2000 in the mandibular nerve (R309, R311). This represents a surplus of up to 100% above normal. Counts of the capped nerves reveal an even greater distal excess, namely 150% for the buccals, and 200% for the mandibular. The latter may be merely a simulated increase due largely, if not wholly, to the doubling back of fibers in capped nerve, resulting in many fibers being counted twice. At any rate, lack of peripheral connections has definitely not caused a reduction of the number of useless fibers.

(3) The fiber spectra of the capped nerves show a spectacular shift toward the small fiber classes. The largest fibers of the capped nerve lie from two to three classes below, i.e., are from 2 to 3 micra (ca. 30%) smaller than, the largest fibers of either a normal or a regenerated nerve with peripheral connections. Nearly three-fourths of all capped fibers fall within the two lowest size classes, i.e., below 2.1 micra, while in connected nerves almost three-fourths of all regenerated fibers measure more than 2.1 micra. These facts are illustrated graphically in figure 3.

The graphs reveal that the spectrum of connected regenerated nerves (upper row) resembles that of normal controls. There is a general size deficit, but, with the exception of case R310, it is slight. In contrast, the spectrum of capped nerves (lower row) is highly deficient, both in comparison to normal controls and to connected regenerates. The uniformity of the three cases in quantitative regards is remarkable.

The last column of table 2 gives for each nerve the total cross sectional areas of all fibers in arbitrary units (arrived at as the sum of the products of numbers of fibers in each class times the square of the average diameter of that class). According to these figures, there is substantially less axoplasm present in the cross section of a capped nerve than in a normal control or connected regenerated nerve, even though the fiber count is much higher. The smaller fiber size is, therefore, not simply the result of a standard mass of axoplasm being distributed over a larger number of fibers, but expresses a real deficit of axoplasm.

Not only the regenerated distal portions of the capped fibers were deficient in size but the proximal old parts had likewise suffered a marked reduction. The graph, figure 4, illustrates this fact. It shows for comparison the fiber spectra at levels distal (full lines) and proximal (dotted lines) to the crush level (R310). It can readily be seen

that the fiber spectrum of the proximal mandibular nerve (level A; fig. 1, III) containing the capped branch, has much more nearly the configuration of the distal regenerated portion (level B) than that of a normal mandibular nerve. The shift toward the small end is not quite as great as at level B, but this difference is accounted for by the fact

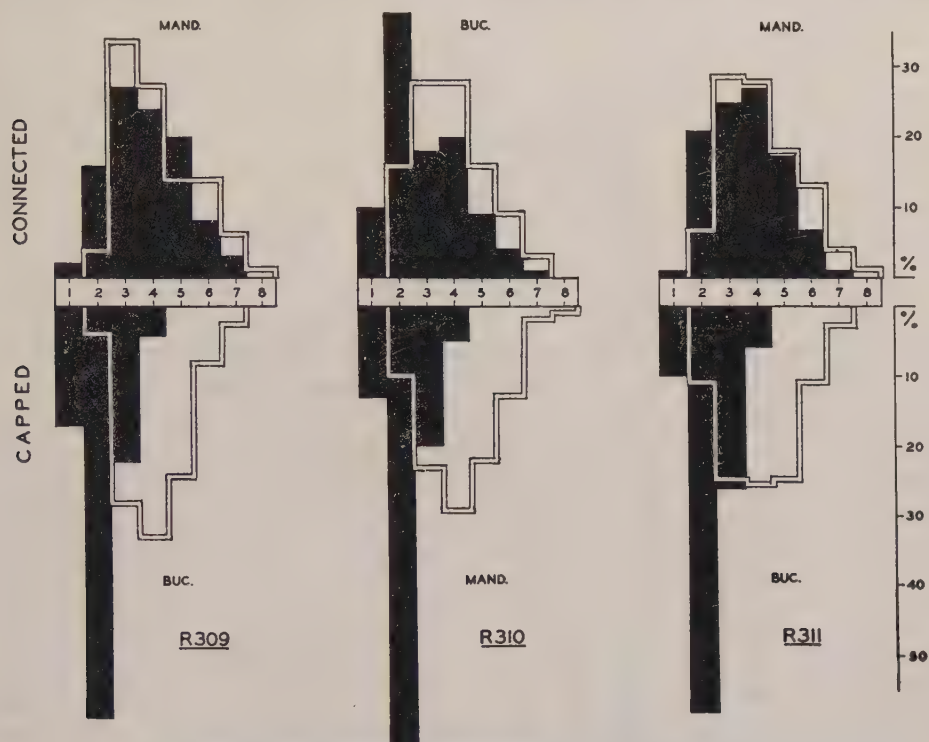


Fig. 3 Graphs of the percentage distribution of fibers according to size in capped and connected branches of the facial nerve (black blocks), and in normal control nerves (blocks with double outlines), 1 year p. op. The fiber spectra of connected regenerated nerves (upper row) approximate those of the controls, while the spectra of the capped nerves (lower row) show a marked shift toward the smallest fiber classes relative to both control and capped nerves. Size class indices express actual diameters as follows: (1) $< 1 \mu$; (2) $1.1-2.1 \mu$; (3) $2.2-3.2 \mu$; 4.4- 5.4μ ; etc. Mand., Mandibular branch; Buc., Buccal branch.

that measurements at level A include ca. 1800 fibers belonging to uncapped branches (cf. fig. 1 and table 2), the presence of which naturally raises the size average. Similarly, the slight difference in the spectra of the connected buccal nerve at level A and a control buccal nerve at level B, seen in the upper half of figure 4, is evidently due to the admixture at the more proximal level of numerous sensory fibers of smaller caliber which leave the nerve between A and B. It is possible,

therefore, that if strictly comparable proximal and distal counts were available, we would find the proximal spectrum to be reduced fully as much as the distal spectrum. How far proximally the deficiency extends, remains undecided, but in view of the long period (1 year) elapsed since the operation, it is reasonable to expect that the whole course of the nerve fiber, including presumably the cell body, has been involved.

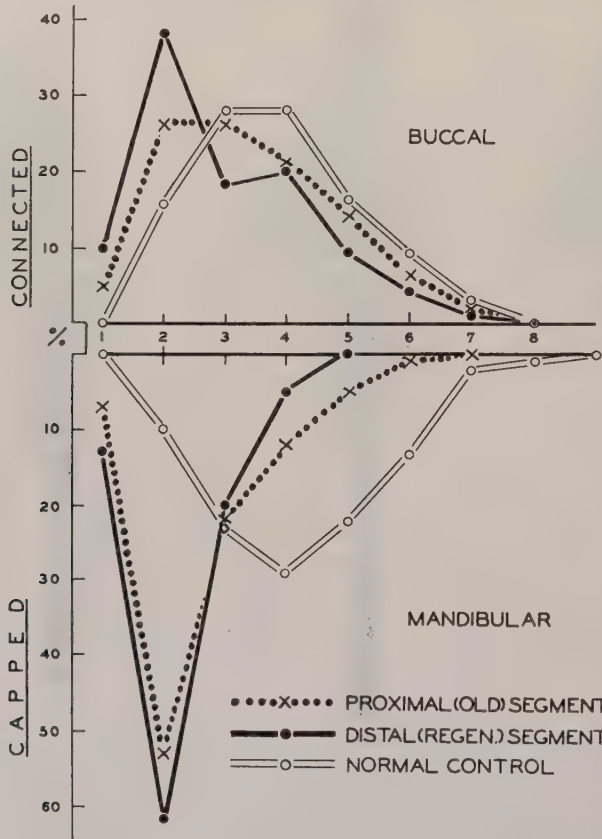


Fig. 4 Graph of the fiber spectra, at levels proximal and distal to original crush, in connected and capped branches of the facial nerve (R310), and control spectra. Both proximal and distal segments of the capped mandibular nerve show similar deficits of fiber size.

DISCUSSION

The results of these experiments thus confirm and amplify the previous observations of Weiss and Taylor ('44 a) that lack of resumption of peripheral connections, while not entailing resorption of the idle branches, stunts their growth in width. The effect of capping is to be viewed against the background of ordinary regeneration after nerve

crushing with restoration of peripheral connections. During such regeneration, some fiber branching has been known to occur, resulting in an overproduction of regenerated fibers. This distal surplus was first noted by Greenman ('13) and, incidentally, has since been recommended for clinical exploitation (Dogliotti, '35; Billig and van Harreveld, '43). A very slight augmentation of fiber numbers after crushing is also indicated in data presented by Gutmann and Sanders ('43) in tables 1 and 3 of their paper, although the authors themselves have not stated this explicitly. In our own cases, crushed nerves with unimpeded regeneration have shown a much more marked increase, up to 100%. Presumably, this is due to the greater length of the crushed zone (a few millimeters), since the number of neurilemmal tubes ruptured in the process of crushing and hence, the incidence of branching during subsequent regeneration, will obviously mount in proportion to the extent of the lesion. This fact may likewise account for a minor discrepancy between our own data and those of Gutmann and Sanders relating to the recovery of the size spectrum in crushed nerves. According to Gutmann and Sanders, the spectrum returns to normal within 250 to 300 days (in the rabbit), while we have recorded a slight residual size deficit even after 1 year (fig. 3, top). The latter could readily be explained by the shunting of severed fibers into foreign paths (see below). Even in our cases, however, the spectrum closely approached that of normal nerve if the fibers were allowed to become reconnected with the peripheral tissues.

In contrast, nerves prevented from effecting peripheral attachments differ from connected ones in three regards: (1) they give even higher fiber counts; (2) the size spectrum of the regenerated portion falls far short of normal; and (3) the old proximal portion undergoes corresponding atrophy.

The number of fibers counted in capped nerves was up to three times as high as in normal nerves, which means an added increase of almost again as much as that recorded in regenerated, but connected, nerves. As mentioned before, it is impossible to estimate how much of this surplus is merely apparent, due to the fact that each fiber which has doubled back at the distal obstruction, passes through the counting level twice. On the other hand, if the distal increase should turn out to be real, it might signify that the fibers of a connected nerve, by continuing to grow in width, would sap, and cause the obliteration of, any of their weaker collateral branches. In capped nerves, this reduction of an initial overproduction would fail to occur simply because fiber growth is less vigorous and, therefore, less competitive.

The basic result of the lack of peripheral connections seems to be just this reduced ability of the fibers to grow in width. The facts in the case, as presented in the text, are so clear and consistent that they need no amplification. Certain implications, however, deserve comment. It has been known that the regenerated portion of a large nerve fiber which has grown into the distal stump of a small one remains undersized (Nageotte and Guyon, '18; Holmes and Young, '42). While this phenomenon has been ascribed to the restraining action of the neurilemmal tubes, and though the throttling effect on fiber growth of an undersized fiber channel has been experimentally demonstrated (Weiss and Taylor, '44 b), our present observations raise the question whether part of the size deficit after the crossing of incongruous nerves might not have to be ascribed to the failure of many of the crossed fibers to acquire functional terminal connections. Sensory fibers, for instance, are definitely incapable of forming transmissive attachments to muscle fibers (Weiss and Edds, '45; Gutmann, '45). Their ends are, in a physiological sense, blind, and this might stunt their growth just as if they were mechanically blocked by capping. Fiber measurements on sensory-motor nerve crosses are under way.² Prior to complete size records it would seem premature to adopt the suggestion advanced by Gutmann ('45, p. 5), that "maturation of nerve fibers is apparently not dependent on the reinnervation of an end-organ but will take place without such a connexion".

The size deficit of capped nerves was equally well marked after 4 months and after 1 year. A direct quantitative comparison between the two series is not feasible because they were carried out with different kinds of nerves. It is safe to conclude, however, that the size differential between connected and capped nerves does not diminish with time and can, therefore, be considered as permanent. Growth of the blocked fibers is not merely retarded; it remains deficient as long as the peripheral disconnection lasts.

The most unexpected result was the observation that this depression of growth was not restricted to the newly formed distal portion of the fibers, but continued into the old proximal part, where it manifested itself as a marked atrophy (fig. 4). Since these nerves had been allowed to regenerate for very short distances only (15 mm. between crush and cap), this atrophy cannot be ascribed to the regeneration process, but must be considered as a direct result of the lack of pe-

² In a preliminary survey of reciprocal crosses between sensory saphenous and motor quadriceps nerves, 3 months after operation, we have noted a marked reduction of fiber diameters in the proximal stumps, as compared with normal control nerves.

ripheral connections. Logically, therefore, the fibers of any transected proximal stump, even when completely prevented from regenerating (e.g. in capped amputation stumps) may be expected to undergo gradual shrinkage in width. Experiments to test this assumption are being carried out. The question of whether the cell body of the atrophying fibers exhibits a corresponding shrinkage is likewise being investigated.

Evidently, the failure of the regenerated axon portion to grow up and the actual decline of the old portion are merely two different aspects of the same phenomenon, namely, a shift in the balance of anabolic and katabolic processes in axoplasm in favor of the latter. That is to say, unconnected nerve fibers differ from connected ones in that they either synthesize less axoplasm or use up more. After becoming connected, either the rate of synthesis increases or the rate of dissipation declines. In either case, surplus begins to accumulate which accrues to the width of the fiber. The causes of this change may be sought either in an active stimulation of the growth of the neuron by an influence received from its end organ, or merely in the fact that the neuron has ceased to roam. The former possibility envisages a "trophic" effect which would be exerted constantly by muscle fibers upon their motoneurons, and by sense organs upon their sensory fibers, and which would sustain the synthesis of neuron substance on an elevated level, thus maintaining a larger caliber. Disconnection would deprive the fiber of this effect and thus lead to progressive shrinkage to the level characteristic of an unconnected fiber.

The notion of some influence passing from the peripheral tissue to the nerve fiber and farther on centrad is not without precedent. According to Weiss (reviews in '36, '41), some such ascending influences determine the specific central relations of a nerve fiber. Furthermore, motoneurons deprived of their peripheral connections by transection undergo marked changes in their physiological state and response (Acheson, Lee and Morison, '42;³ Campbell, '44). Chromatolysis following nerve injury might likewise turn out to be due to the loss of some peripheral influence rather than to the direct traumatic effect of the lesion. It remains to be decided, however, whether and how closely any of these phenomena are related to the size effect described in this paper.

As for the latter effect, it seems indicated to keep in mind the other alternative mentioned before, namely, that the fact of peripheral con-

³ Acheson, Lee and Morison, studying proximal phrenic nerve stumps oscillographically at various intervals after transection, noticed that conduction velocity in such nerves was slower than in controls, but left the fact unexplained. Our present results indicate that this decline of conduction rate is directly attributable to diminished average fiber size.

nection may imply nothing more for a fiber than that its state of unrest has come to an end. Conceivably, a considerable amount of energy has to be expended by an axon to keep its free tip in motion. Terminal attachment to another tissue would make this energy available for other purposes, e.g., increased synthesis of axoplasm. A free tip need not actually advance in order to be rated as in motion. There is presumably incessant protrusion and retraction of filopodia, but whether this results in elongation of the fiber or merely in stationary idling depends entirely on the physical constitution of the surroundings (Weiss, '44 c), and the effort spent by the fiber may be as great in one case as in the other. An arrested fiber in a capped nerve may, therefore, be forced to expend more energy than a connected fiber even though it makes no perceptible advance. That ceaseless agitation along the free surface of cells is suppressed by surface contact with other cells of conforming character, has recently been demonstrated by Holtfreter ('43), and many facts suggest a similar concept for the relation between the nerve fiber and its end organ. In this view, a disconnected axon would atrophy because energy formerly available for metabolic reconstitution of its mass would now be partly consumed in the activity of its free end. But only further experimentation can bring a decision between the two alternatives set forth in the preceding discussion.

A third possibility, namely, that a proximal axon stump shrinks merely because its substance "flows" out to form the regenerating distal segment, can be discounted. This interpretation has been advanced by Gutmann and Sanders ('43), who had observed that the fibers in the proximal stump of any regenerating nerve suffer some reversible loss of diameter. Weiss, recognizing the primary advance of the axon tip as a purely physical elongation, i.e., redistribution of preexisting, rather than production of new, axon substance, has adopted this argument in a recent review (Weiss, '44 c). It now seems, however, that the facts must be reinterpreted. The volume of regenerated axoplasm in capped nerves is only a small fraction of that of connected nerves; not only is the regenerated segment in the former very short, but it also has a much smaller cross sectional area (table 2). Therefore, if the proximal fiber stump were to lose diameter merely as a result of the draining of its substance into the distal process, the connected nerves should show a much greater loss than the capped ones. Since quite the opposite is true, and since, furthermore, capped nerves remain permanently undersized, the reduction of fiber diameter signifies actual atrophy, that is, loss of substance, rather than mere redistribution with elongation at the expense of width. This is not to deny the

reality of this latter process. The young regenerating tip is undoubtedly nothing but axoplasm siphoned from the stem of the fiber by interfacial tensions (Weiss, '44 c). However, the stem is so thick and the new part so slender, that the diminution of the former could hardly be of the observed magnitude. The newly spun out part usually measures less than 1 micron across and thus has a cross section of less than one twenty-fifth of that of an average fiber stem of 5 micra. For each centimeter of distal advance, 1 cm. of the proximal stump would, therefore, yield about one twenty-fifth (4%) of its mass, which would correspond to a reduction in diameter of only 2%. This difference would be barely measurable. Thus, though we must postulate some loss of proximal axoplasm in the process of elongation, this can, at best, account for but a fraction of the total shrinkage, the rest being a matter of true atrophy.

It is significant to note that according to our results, the "trophic" relation between nerve fibers and terminal tissues is mutual and that the integrity of myoneural connection is as essential for the trophic maintenance of the nerve fiber, as it is for the muscle fiber.

A moderate atrophy such as occurs regularly in the proximal parts of regenerating nerve fibers during the period between severance and reconnection with end organs, seems to be fully reversible (Gutmann and Sanders, '43). It is conceivable, however, that with increasing periods of disconnection, and accordingly higher degrees of atrophy, the recuperative ability of the axons would decline. If this should prove to be the case, it would present another argument against prolonged delay in the clinical repair of injured nerves.

SUMMARY

Ordinary nerve regeneration, in which the nerve fibers were allowed to reestablish terminal connections ("connected" nerves), was compared with regeneration in blind stumps, where the fibers were prevented from connecting with peripheral tissues ("capped" nerves).

After regeneration periods of 4 and 12 months, the fiber size spectra of the capped nerves differed markedly from those of the connected nerves. The regenerated portions of the connected nerves have recovered nearly normal fiber size spectra, while the capped fibers have remained undersized. In three cases measured 1 year postoperative, an average of 71% of all fibers of the connected regenerates were larger than 2.1 micra, while 73% of all fibers of the capped nerves had diameters below that value. The largest fibers of the connected nerves were from 2 to 3 micra larger than the largest fibers of the capped nerves.

The old proximal portions of capped nerve fibers underwent marked reduction of diameter. There was, however, no sign of resorption of such fibers. On the contrary, fiber counts in capped nerves gave higher totals than those of connected regenerates. This surplus may be due to the doubling back of fibers at the occluded nerve end.

The deficit of fiber size in both the old and new portions of a fiber disconnected from its terminal tissue results from atrophy, due either to the loss of some "trophic" influence exerted by the peripheral tissues, or to excessive energy demands by the free and active fiber tips.

The results demonstrate the reciprocity of "trophic" relations between nerve fibers and terminal tissues.

ADDENDUM

After completion of this manuscript, a brief note by Sanders and Young appeared in *Nature* (vol. 155, p. 237, 1945), which confirms the effect of peripheral connection on the diameter of the regenerated portions of nerve fibers described in this paper. The note fails to mention atrophy of the old fiber portions.

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THE ETIOLOGY AND INHERITANCE OF INEQUALITIES IN THE JAWS OF SHEEP

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NINE FIGURES

Morphologic misfits in the jaws of experimental and domestic animals have been observed for some time. The two structures involved, namely the upper and lower jaws, or the maxilla and mandible, respectively, are so closely coordinated in function that any appreciable inequality in their development interferes with the ability of the animals to obtain and chew their food. In earlier studies of inequalities in the jaws of sheep it was pointed out that failure of structural harmony has a marked influence on the maximum usefulness of these parts for apprehending and chewing their food and the result is decreased production (Nordby, '31).

Efficiency would appear to be in direct proportion to the degree of developmental harmony in the two jaws (figs. 1-2). Except for some breeds of pet dogs, which have been bred for marked restrictions in the length of the upper jaw, man in general is selecting for usefulness. Natural selection favors correlated rather than independent changes in the jaw which do not conform to the efficiency pattern.

The most common defects are those which involve unequal lengths in the jaws giving rise to brachygnathia, commonly referred to as an "overshot" condition, in which the lower jaw appears too short, and prognathism or "undershot", in which the lower jaw appears too long (figs. 3-5). Both of these definitions describe the apparent condition, and imply that the defect is limited to the mandible. Such an inference is not justified in the light of available information (Nordby, '35). Both jaws might vary in the same direction and be uniformly longer or shorter than the normal length for the breed. In either case they would be considered normal for dental occlusion. Brachygnathia may be the result of a normal maxilla and a short mandible or of a normal

¹ Respectively, Director, animal husbandman, associate animal husbandman and animal husbandman, Western Sheep Breeding Laboratory and U. S. Sheep Experiment Station, Bureau of Animal Industry, Dubois, Idaho.

mandible and long maxilla. Both members may also vary in opposite directions to form the defect. Prognathous jaws may be due to a normal mandible and a short maxilla, a long mandible and a normal maxilla, or exaggerated development in the mandible and underdevelopment in the maxilla (figs. 1-5). These may be due to Mendelizing variations in each of the elements and are independent of any fixed pattern of growth association that may obtain. Stockard ('41) has found in crossing breeds of dogs, which have different shapes and types of jaws, that the independence in development of maxilla and mandible is brought to light, and adds that natural selection has brought about a structural and functional association between the jaws which largely conceals this independence.



Fig. 1 Normal occlusion of the jaws in sheep.



Fig. 2 Relation of incisors and premaxilla in skull with normal occlusion.

The conventional definitions of these two general defects therefore define the apparent and not the actual condition. It would be difficult to assign all of these cases to a common source of genic or environmental influence, because it is not very probable that a common influence would materially affect one and not the other of the two closely coordinated parts, or that it would affect them in opposite directions. To whatever extent general genes may be involved in the tissue differentiation of the maxilla and mandible it would seem reasonable to expect that they would function with a fair degree of uniformity in both members.

Structural disharmony has been found to be environmental as well as genic. Workany and Nelson ('40, '41, '42) and Workany, Nelson and Schraffenberger ('43), in a study of the influence of restricted diets on

the progeny of rats, were successful in inducing congenital abnormalities in many parts of the skeleton. These investigators found that the corpus mandibulae was reduced in length while the ramus was not severely affected. They also found that the maxilla was usually normal, except for six cases in which a reduction in size was associated with extreme shortness of the mandible. Considerable variation was observed in the magnitude of the defects in the mandible as well as in many other bones of the body.



Fig. 3 Morphologic misfits in jaws. The incisors in extreme cases register against the palate back of the dental pad. The overshoot defect.



Fig. 4 A pronounced case of disharmony between the premaxilla and incisors.



Fig. 5 An undershot condition in which the incisors extend beyond the dental pad.

Variations in the magnitude of morphologic jaw inequalities and their genic origin in sheep have been previously reported (Norby, '31, '35). Postnatal changes have also been described, and general recommendations have been made for eliminating the defect in sheep. While no attempt was made in the earlier work to incriminate special genes in kind or number, it was pointed out that the apparent confusion in the etiology of the defect precluded the assignment of the cause to any single structure or part of a structure — a situation which appeared to involve both general and specific genic potentials. Although the very close co-ordination in function of the maxilla and mandible suggests a common genic potential, the substantial number of defective cases on record appears to be ample evidence that independent genic influence does exist.

The developmental history of the mandible in vertebrates is complicated. This would suggest, not only that there are independent genic potentials involved but, likewise, that they are complicated. Specific variations in the mandible pattern at the distal, and apparently also at the proximal end processes are, no doubt, influenced in their ontogenic differentiations by modifying genes which cause disturbances great enough to inhibit or exaggerate normal development. From the work of Workany and Nelson ('41) with rats it would appear that the mandible is definitely more sensitive to environmental restrictions than is the maxilla, because pronounced defects occur in the mandible much more frequently than in the maxilla. On the basis of the general concept in embryology that the two jaws originate from closely related branchial structures in the first branchial arch, and differentiate into functionally coordinated parts, one might expect a closer agreement in their reactions to stimuli that modify development than that which is reported by these authors. This basic contribution, however, does suggest less genic stability in the mandible than in the maxilla in rats. It also suggests independence in ontogenic differentiation and may be a cause for caution in assuming too much general genic action that has a common influence on the upper and lower jaw.

In his study with dogs Stockard has concluded that modifications in the palate and the maxilla are more largely responsible for dental malocclusion than are changes in the width-length relations of the mandible. And, in bulldog malocclusions, the upper jaw is the decidedly modified part; the lower jaw is much less changed in its proportions, and therefore it projects forward beyond the shortened upper facial region. Stockard has concluded from these studies that in dogs, the responsibility of mandibular deformity for dental malocclusion is largely secondary.

Stockard has found in the Dachshund-Pekinese and other crosses that the genetic basis for the pattern of the maxilla is entirely distinct and independent of the genetic background for the mandibular size and form. Also, the genetic constitution of the individual influences the patterns of the two jaws separately in spite of the internal environment as influenced by the endocrine glands.

The etiology and inheritance of these defects in sheep appear to have some things in common with their behavior in dogs and rats. Stockard postulates partial dominance and a complex genetic basis of the factors determining either long or short jaws from the crosses of short and long muzzled breeds, and that the reduction in length of each jaw is influenced by a complex of factors, some of which are dominant and some recessive to the normal jaw length.

In an effort to get specific information that might contribute further to the etiology of the problem in sheep and to study further the genetic causes of dental malocclusions, a project was undertaken to compare dimensions of normal and defective skulls, and to make further matings of defective animals or of animals related to defective animals. Information was also needed on the change of the defect with age and its influence on viability and other traits. Finally specific information was needed as to the best method of selective breeding to eradicate the defect.

MATERIALS AND METHODS

The particular cranial structures and parts thereof which were suspected of being involved in the development of jaw inequalities were measured in both normal and abnormal animals to identify as nearly as possible the specific structural changes responsible for the defect. The linear measurements of the skull, maxilla, premaxilla and mandible indicated in figure 6 were taken with a long-jawed metric caliper designed for the purpose. Inasmuch as variations in the angle of the mandible or incisor teeth appeared capable of obscuring or exaggerating jaw inequalities, special instruments were designed for the purpose of measuring them. Since these instruments are well adapted to the measurement of angles in other skeletal structures, the principles of their construction and use are illustrated in figures 7, 8 and 9.²

Both normal and abnormal skulls were obtained from animals born in the Rambouillet flocks of the Western Sheep Breeding Laboratory, so that wide differences in previous management and general genetic background would be minimized. The abnormal sheep were excess ani-

² These devices were developed by the senior author while employed by the University of Idaho.

mals from the genetic experiment which was initiated in 1937 with overshot animals found in the flock at that time. An effort was made to make available those animals for anatomical study which showed the greatest visible degree of jaw inequality while still alive. The choice of normal ewes was determined largely by their availability at a time when the work could be done, with the requisite that the incisors register normally with the dental pad.

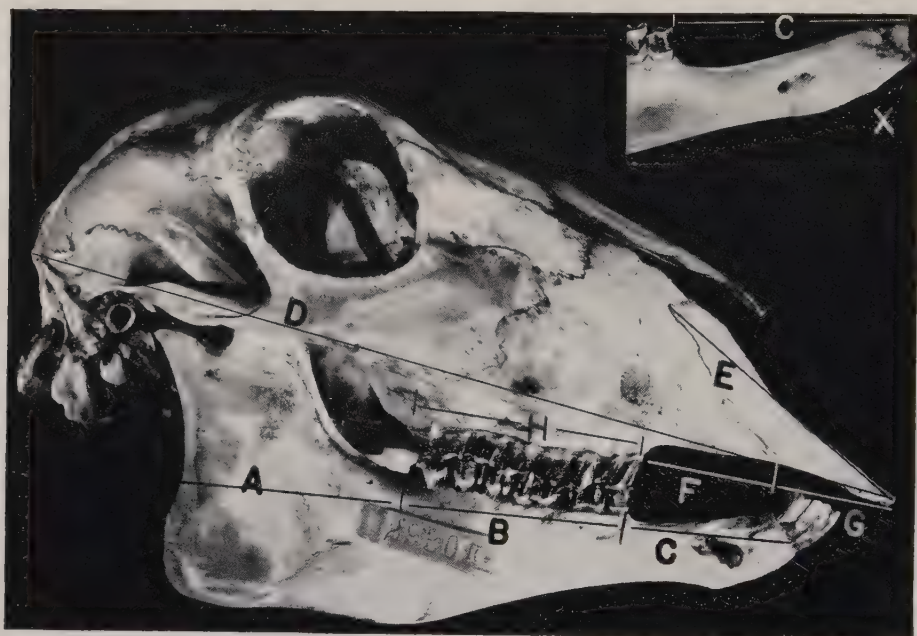


Fig. 6 Cleared skull of overshot ewe indicating the various measurements taken.

ABC, length of mandible

A, proximal end of mandible

B, molar space of mandible

C, interdental space

D, total length of skull

E, total length of premaxilla

F, length from molars to distal end of premaxilla

G, body of premaxilla

H, molar space of maxilla

Insert X shows specific method of determining length of C.

Inasmuch as the visible inequality in jaws has been observed in some cases to change from birth to maturity, it appeared that some clues as to its developmental history could be obtained by studying lambs as well as mature animals. The only lambs available for this purpose were some overshot ram lambs about 8 months of age.

The heads were steamed in an autoclave to facilitate the removal of flesh, cleared, and measured as described above. In all, the skulls of fifteen normal mature ewes, nineteen overshot ewes and nine overshot ram lambs were studied.

MEASUREMENTS

The detailed measurements and the averages for each group of animals are given in table 1. That the overshoot ewes differ markedly in anatomical structure from the normal ewes is immediately apparent in the averages for the two groups. In the overshoot ewes, the mandible averages 1.0 cm. shorter and the skull averages 0.8 cm. longer than in

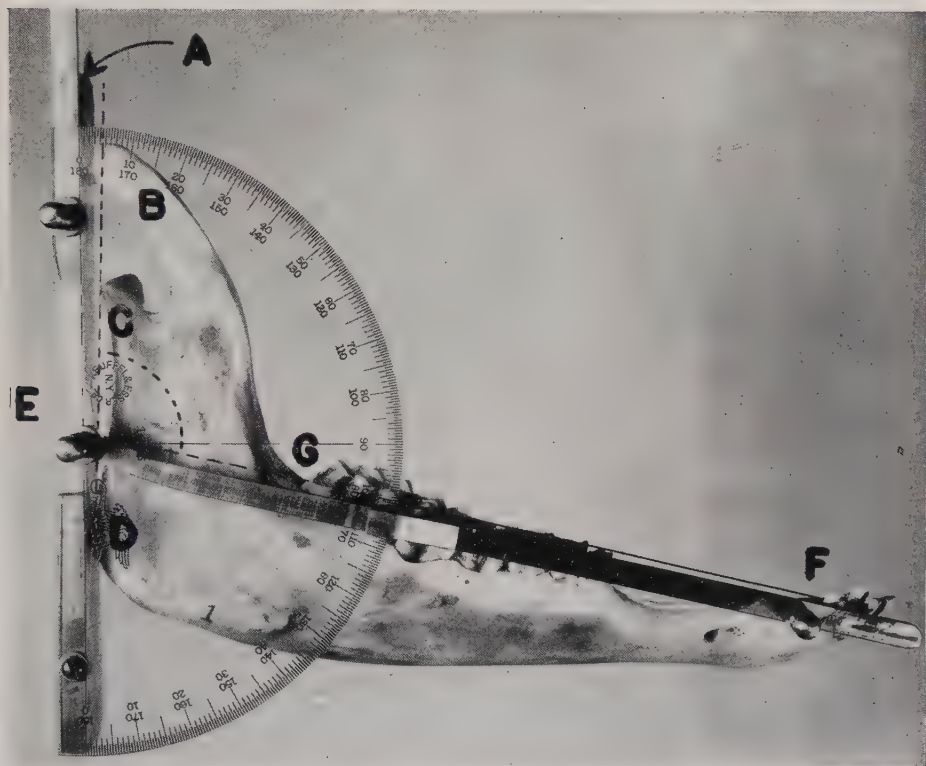


Fig. 7 Device for measuring angle of mandible.

E, supporting frame of caliper
 B, coronary process of mandible
 C, condyle
 D, ramus

F-G, points upon which angle was based
 A, slot cut in base of device to accommodate coronary process and allow base of device to rest against condyle.

normal ewes. Since the differences are in opposite directions the total disproportion in the jaws of the overshoot group amounts to 1.8 cm. This difference may be compared with the average jaw inequality of the overshoot ewes, which is 1.2 cm. as measured when the animals were alive. The skulls in the normal ewes average 6.7 cm. longer than the mandibles, while the differences are 8.5 and 8.3 cm. in the overshoot ewes and ram lambs, respectively.

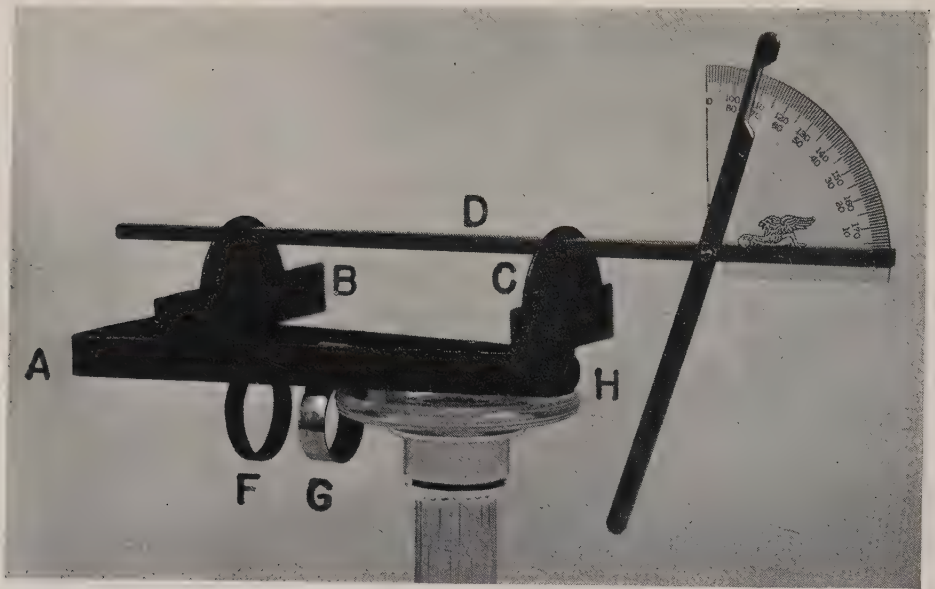


Fig. 8 Device used for measuring angle of teeth.

- | | |
|---|---|
| A, frame | G, stationary with frame for holding device in position |
| B, movable support of caliper frame D | H, small rod over which B moves for adjusting space between B and C |
| C, stationary support for caliper frame D | |
| D, caliper frame | |
| F, part of movable support B | |



Fig. 9 Application of device in figure 8 for measuring angles of incisors. Note position of index finger of left hand which holds device in position by means of F and G in figure 8.

The ratio for mandible length to skull length ($\frac{ABC}{D}$ in table 1) reflects the severity of the defect more clearly than any single measurement. The ratios for normal ewes do not overlap with those for overshot ewes or ram lambs, all of the normal ewes being 71% or higher and those in the overshot groups 70% or lower.

Each of the three regions into which the mandible was divided is shorter in the overshot group of ewes than in the normal group. The interdental space is shorter by 0.49 cm., or by 9% of its average length in normal skulls; the molar space (B) is shorter by 0.14 cm., or by 2%, while the proximal end of the mandible (A) is shorter by 0.41 cm., or by 7%. At first appearance these differences may seem to indicate that the proximal and distal ends of the mandible are more severely affected than the central portion. This interpretation may be a misconception, however, because the divisions do not correspond to any particular anatomical sections of the mandible, since in each case they are limited by the space occupied by the molar teeth. A more correct interpretation seems to be that the mandible, reduced in overshot ewes by 5.6% of the normal length, is more severely affected by the overshot condition than is the molar space, which is reduced by only 2%.

The different sections of the upper jaw are affected somewhat differently than those of the mandible. The body of the premaxilla (G) is delineated at a definite anatomical point where the distal end of the maxilla articulates with the body of the premaxilla. The body of the premaxilla does not seem to be lengthened appreciably by the overshot condition, as it is only 0.01 cm., or 0.5%, longer than in normal ewes. This may be reasonable inasmuch as the premaxilla and maxilla arise from separate ossification centers. The distal end of the maxilla (F) is extended considerably, however, since in overshot ewes it averages 0.46 cm., or 12%, longer than in normal ewes. The molar space of the maxilla (H) is 0.20 cm., or 3%, longer than normal. The remaining structures concerned in length of skull are evidently little affected by the overshot condition. Hence, the maxilla is the only structure of the upper jaw that is appreciably longer in overshot ewes than in normal ewes. As with the mandible, the maxilla is affected much more than is the molar space.

The molar teeth of the two jaws were found to register normally and to be worn uniformly at the distal and proximal ends of the molar spaces (B and H) in both normal and overshot ewes. The molar spaces are relatively shorter in the ram lambs than in the ewes because all of their molar teeth had not developed. In other respects the data on the ram lambs confirm the differences between overshot and normal ewes.

TABLE 1
Dimensions and angles of various skull structures of Rambouillet ewes and
ram lambs with normal and abnormal jaws.

IDENTIFICATION NO.	INEQUALITY OF JAWS IN LIVE ANIMAL	LENGTH OF MANDIBLE	INTERDENTAL SPACE	MOLAR SPACE OF MANDIBLE	LENGTH OF SKULL	LENGTH FROM MOLARS TO DISTAL END OF MAXILLA			MOLAR SPACE OF MAXILLA	TOTAL LENGTH OF PREMAXILLA	BODY OF PREMAXILLA	ANGLE OF MANDIBLE	ANGLE OF INCISORS		
							ABC D	O F					Proximal	Distal	
	cm.	cm.	cm.	cm.	cm.	cm.	per- cent	per- cent	cm.	cm.	cm.	degrees	degrees	degrees	
<i>Normal mature ewes:</i>															
8772	19.5	5.9	7.7	25.7	4.1	76	144	7.8	8.0	2.7	98	110	90		
7142	17.9	5.1	6.4	24.2	3.6	74	142	6.2	6.8	2.7	93	110	90		
8830	19.5	6.0	7.7	26.5	4.2	74	143	7.3	8.4	2.9	99	113	93		
3080	18.3	5.5	7.2	24.5	4.5	75	122	6.2	7.4	2.8	94	110	89		
2190	18.5	5.0	7.4	25.0	3.3	74	152	7.6	8.3	2.9	98	114	88		
2734	19.8	6.2	7.2	27.2	4.1	73	151	7.4	8.3	3.0	99	105	90		
1242	18.7	5.3	7.2	25.0	3.8	75	139	7.1	8.3	2.9	96	102	90		
2980	19.2	5.9	7.2	25.9	3.6	74	164	7.2	8.1	3.1	96	110	85		
3006	17.9	5.3	6.9	24.1	3.5	74	151	6.8	7.3	2.7	97	125	93		
3758	18.1	5.5	7.1	24.6	3.9	74	141	7.1	7.5	2.6	98	115	88		
4172	19.1	5.4	7.7	24.6	3.6	78	150	7.4	7.7	2.9	94	125	94		
4158	18.5	5.0	7.3	26.0	4.1	71	122	7.2	8.3	2.9	96	140	115		
4041	18.6	4.9	7.7	25.3	3.3	74	148	7.4	7.5	2.8	95	127	95		
1800	18.5	5.1	7.7	26.2	3.3	71	155	7.4	7.6	3.0	94	120	95		
3694	18.9	5.2	8.4	26.0	3.4	73	153	8.2	8.0	2.9	103	125	93		
4657	18.4	5.4	7.3	25.1	3.8	73	142	7.3	8.0	2.7	96	118	94		
Average	18.7	5.4	7.4	25.4	3.8	74	145	7.2	7.9	2.8	97	117	93		
Coefficient of variation	3.1	7.9	6.0	3.5	9.8	2.3	7.6	7.0	5.9	4.8	2.6	8.3	7		
<i>Overshot mature ewes:</i>															
45	1.5	17.2	5.0	6.2	26.0	4.1	66	122	6.7	7.7	2.5	103	105	90	
46	1.2	17.2	5.2	7.3	25.2	4.1	68	127	7.8	7.8	2.9	103	118	95	
2827	1.3	18.9	5.1	7.5	28.0	4.0	68	128	7.4	8.9	3.3	102	113	94	
8518	1.1	18.2	5.4	7.0	26.4	4.5	69	120	7.5	7.6	2.4	101	112	93	
3913	.4	17.5	4.7	7.4	25.4	3.9	69	121	7.1	7.9	3.3	96	125	110	
2880	.7	18.6	5.3	8.3	26.8	4.2	69	126	8.3	7.7	2.9	93	125	100	
1951	.9	17.2	4.8	7.7	25.6	3.7	67	130	7.7	7.4	2.8	97	128	108	
5153	2.0	16.0	3.7	6.4	24.7	4.0	65	93	6.8	7.8	2.6	93	125	105	
2420	.7	18.3	5.1	7.1	27.0	4.4	68	116	7.2	9.0	3.0	96	110	94	
2849	2.1	17.2	4.6	7.7	26.6	5.1	65	90	7.9	8.6	2.7	94	125	90	
2263	.7	18.0	5.7	7.1	26.6	4.3	68	133	7.3	8.0	2.9	98	125	93	
4142	.9	18.1	5.1	7.3	26.4	3.9	69	131	7.6	7.6	2.9	97	126	97	
3855	.9	17.8	4.9	7.3	25.6	3.6	70	136	7.2	7.4	3.2	94	122	93	
3317	1.1	18.4	5.2	7.7	27.3	4.6	67	113	7.8	8.1	2.8	94	127	95	
6190	.8	17.0	4.4	7.2	25.0	4.0	68	110	7.3	7.4	2.7	98	128	100	
2113	2.1	18.6	5.5	6.9	28.4	5.2	65	106	7.8	8.9	2.8	87	120	90	
6193	1.6	17.7	4.4	8.0	27.4	4.8	65	92	8.2	8.5	2.9	95	130	90	
6195	1.0	16.7	4.5	6.6	24.7	3.8	68	118	6.9	7.6	2.8	96	120	93	
3868	1.0	17.1	5.1	6.8	24.5	3.9	70	131	6.6	8.3	2.9	95	118	92	
Average	1.2	17.7	4.9	7.2	26.2	4.2	68	118	7.4	8.0	2.8	96	121	96	
Coefficient of variation	4.3	9.5	7.3	4.6	10.7	2.5	12.0	8.8	6.7	8.1	4.1	5.7	6		
<i>Overshot ram lambs 8 months old:</i>															
4665	1.0	14.6	4.1	5.0	22.8	3.4	64	121	5.5	6.3	2.5	103	114	95	
4588	1.1	14.4	4.1	5.1	22.4	3.3	64	124	5.6	6.5	2.5	104	114	100	
1379	.8	15.1	4.3	5.6	23.6	3.4	64	126	5.9	6.7	2.7	95	125	101	
5554	.8	14.8	4.0	5.1	23.0	3.4	64	118	5.4	6.9	2.4	102	135	108	
6148	1.2	15.4	4.5	5.1	22.4	3.8	69	118	5.2	7.4	2.7	96	124	103	
5914	1.1	15.2	4.4	5.2	24.5	3.6	62	122	5.4	6.9	2.6	95	120	100	
1202	1.1	15.5	4.5	5.2	24.7	3.2	63	141	5.6	7.0	2.9	108	128	100	
6132	1.0	15.5	4.5	5.4	23.9	3.6	65	125	5.7	7.3	2.7	100	130	102	
7020	1.2	13.3	3.4	5.4	21.3	3.4	62	100	5.1	5.6	2.2	98	116	90	
Average	1.0	14.9	4.2	5.2	23.2	3.5	64	122	5.5	6.7	2.6	100	123	100	
Coefficient of variation	4.7	8.5	3.7	4.7	5.2	3.3	8.7	4.5	8.2	8.0	4.4	6.1	5		

Since the interdental space (C) and the maxillary space (F) were most affected in the overshoot ewes, it might be expected that the ratio C/F would provide the greatest distinction between overshoot and normal animals. This is indeed the case except for two normal ewes where the ratio was as small as in the overshoot animals.

Greater relative variability, as indicated by the coefficients of variation in table 1, is evident in the linear measurements for the two overshoot groups than for the normal group. This may reflect a greater general disharmony in skeletal proportions due to the overshoot condition.

The correlation coefficients given in table 2 indicate some of the differences in structural relations within each of the groups. Although the overshoot animals had longer skulls and shorter mandibles than the normal ewes, the relationship between skull and mandible length within each group is positive and of about the same magnitude. The relationship between the two molar spaces is slightly higher than is the relationship between skull and mandible length in the two groups of ewes. The association between C and F is somewhat lower, especially in the two overshoot groups. The total length of premaxilla (E) is positively correlated with skull length in the three groups. The size of these correlations indicates that general factors are important in determining variations in the different skeletal structures. This seems to be as true in the overshoot groups as in the normal group, despite the structural disharmony evinced by the jaw inequality.

The coefficients of variation for the angle of the mandible (fig. 7) given in table 1 are considerably higher for the two overshoot groups than for the normal ewes, which indicates that the angle might have been disturbed by the overshoot condition. However, there is no indication of significance between the average angles for the three groups. Correlation coefficients between the angle and the ratio $\frac{ABC}{D}$ were small and inconsistent (table 2), indicating that the angle was not influenced by the relative length of the two structures. Thus there is no trustworthy evidence that the angle of the mandible as measured here was influenced by the overshoot condition.

The negative correlation between the ratio $\frac{ABC}{D}$ and the distal angle of the incisor teeth (fig. 9) in normal ewes indicates that a tendency exists in these sheep for the angle of the teeth to change with the relative length of the mandible and skull. In this case, relatively long mandibles are associated with more perpendicular growth of the incisor teeth toward the dental pad, indicating that the angular inclination of the teeth varies to compensate for the jaw inequalities in normal

sheep. Since the correlation between $\frac{ABC}{D}$ and the angle of the incisors are positive for both overshoot ewes and ram lambs, no compensating tendency is apparent in overshoot animals. Similar relations seem to be indicated by the correlations involving the proximal angle of the incisors also. Furthermore both the proximal and distal angles of the incisors are much less variable in the two overshoot groups than in the normal group, whereas the skeletal structures are less variable in the latter (table 1). The average angles of the incisor teeth in the overshoot groups were very similar to those of the normal groups.

TABLE 2

Correlation coefficients between different skeletal measurements in normal ewes, overshoot ewes, and overshoot ram lambs.

SKELETAL MEASUREMENTS	CORRELATION COEFFICIENT BETWEEN INDICATED MEASUREMENTS FOR		
	Normal ewes	Overshoot ewes	Overshoot ram lambs
ABC and D	.77 ²	.80 ²	.80 ²
B and H	.83 ²	.84 ²	.42
C and F	.59 ¹	.22	.33
E and D	.68 ²	.62 ²	.61
ABC/D and angle mandible	— .14	.28	— .19
ABC/D and distal angle of incisors	— .47 ¹	.16	.22
ABC/D and proximal angle of incisors	— .32	— .13	.45

¹ Signifies probability of chance occurrence < .05.

² Signifies probability of chance occurrence < .01.

It is obvious that the amount of jaw inequality which can be compensated for by the variation in the angle of the incisors is limited by the length of the teeth to relatively small inequalities. The cause for the compensatory tendency in normal sheep may be that the pressure exerted by the dental pad on the incisors varies with relative mandible and skull length. When the degree of inequality becomes so great that the incisors do not register with the dental pad, then the angle of the incisors is determined by other factors. The more perpendicular angle of the incisors shown in the undershot animal (fig. 5) as compared with the normal (fig. 2) is commonly observed in living animals.

DESCRIPTION AND RESULTS OF EXPERIMENTAL MATINGS

In the course of inspection of range Rambouillet sheep at the U. S. Sheep Experiment Station, Dubois, Idaho, in the fall of 1937 it was found that 24 out of about 1500 Rambouillet ewes had definitely overshoot jaws. That is, the upper jaw protruded over the lower jaw by at

least 0.3 cm. The average difference between the upper and lower jaws for these ewes was 0.7 cm. with a maximum of 1.8 cm. One Rambouillet ram was available which was about 1.0 cm. overshot. It was decided to use these defective animals for initiating experimental matings to learn more about the inheritance of the defective condition. As the experiment progressed other normal and defective animals were added to the project.

The jaws of all sheep at the Western Sheep Breeding Laboratory and U. S. Sheep Experiment Station since 1937 have been inspected at birth, weaning time (about 130 days), yearling age (about 400 days) and in the fall of the year thereafter. In general, they have been classified according to the proximity of the front teeth with the dental pad as follows: Normal—front teeth and dental pad approximately meet (figs. 1 and 2); slightly overshot—lower jaw slightly shorter than upper; overshot—lower jaw from 0.2 to 0.5 cm. shorter than upper; badly overshot—lower jaw more than 0.5 cm. shorter than upper (figs. 3 and 4). Actual measurements were usually made on all defective animals at weanling and yearling age using specially designed jaw measuring calipers (Nordby, '35). For the purpose of this report all sheep classified as "slightly overshot" have been considered normal and all sheep classified as "overshot" or "badly overshot" or those with lower jaws shorter than the upper jaws by 0.2 cm. or more have been considered defective. When observations made at different ages have varied, the animal has been classified according to the most defective measurement.

Comparison of viability and other traits for normal and defective lambs in an experimental group

The influence of the defect upon viability is shown in table 3. The numbers of lambs weaned and lambs dead before weaning for normal and defective lambs agreed very closely with those expected upon the hypothesis that the defect has no influence on viability up to weaning age. Lambs included in table 3 had at least one defective parent.

TABLE 3

Numbers of normal and defective lambs weaned and dead before weaning.

TYPE OF ANIMAL	WEANED	DEAD BEFORE WEANING	TOTAL
Normal	184	70	254
Defective	39	11	50
Total	223	81	304

Table 4 shows the average birth weights, weaning weights and staple length of wool for normal and defective lambs. Normal lambs were 0.34 pound heavier than defective lambs at birth but this difference was not statistically significant. The difference at weaning was 7.57 pounds, which was highly significant. It appears that defective lambs may be lighter at weaning age because of inability to eat as well as normal lambs, although there may be a genetic association between the defect and smaller body size. Normal lambs in the experimental group had 0.12 cm. longer staple than the defective lambs at weaning age, but the difference was not statistically significant. The defective lambs were more highly inbred than their normal relatives, reflecting the concentration of inheritance from rams known to transmit the defect, and the difference in inbreeding alone would account for some of the difference in weight and staple length in the two groups.

TABLE 4
Average performance for normal and defective lambs at weaning age.

ITEM COMPARED	NORMAL	DEFECTIVE	N — D	P
Birth weight (lbs.)	10.01	9.67	0.34	> .05
Weaning weight (lbs.)	71.59	64.02	7.57	< .01
Staple length (cm.)	2.46	2.34	.12	> .05
Inbreeding (percent)	8.98	15.02	6.04	

Influence of age on degrees of defectiveness

Thirty-two of the overshot lambs were measured for degree of overshotness at birth and at weaning age. The average degree of overshotness increased from 0.50 to 0.98 cm. during this period. Seven of the thirty-two lambs were measured also at yearling age and they showed a further increase in the degree of overshotness. The individual records showed a number of lambs rated normal or slightly overshot at birth which became definitely overshot by weaning age. There was no reliable evidence that any of the definitely overshot lambs became more normal later, whereas considerable evidence pointed to increasing severity of the defect from birth to maturity.

Experimental matings

Two related defective rams (3408R and 3872R) have been used most extensively in these matings, while 4265W, a defective son of 3408R, was used 1 year. In 1942, 2560W, a normal ram which had produced overshot offspring when mated to his normal half sisters, was bred to

sixteen overshot ewes and twenty-three normal ewes, the latter being daughters of 3408R and 3872R. The classification of offspring by years from normal and defective dams mated to these sires is given in table 5. The average frequency of 16.4% defective offspring from these matings may be compared with that of 1.4% defective offspring among nearly 7,000 Rambouillet lambs from normal matings during the same period.

The normal sire of 3872R when mated to normal daughters produced seventeen normal and three defective offspring. This is similar to a 7 to 1 ratio which suggests simple recessive inheritance of the defect. Table 6 gives the results of mating the three defective rams (3408R, 3872R and 4265W) to their daughters, to less closely related ewes and to unrelated ewes. The results from the mating of defective rams to normal relatives and normal non-relatives do not refute the above hypothesis. However, normal daughters mated to their defective sires

TABLE 6
Influence of close mating on frequency of the defect.

RELATIONSHIP OF DAMS TO SIRE	NORMAL DAMS		DEFECTIVE DAMS		Total
	Normal offspring	Defective offspring	Normal offspring	Defective offspring	
Daughters	28	5	10	12	55
Relatives	69	6	53	16	144
Non-relatives	10	0	48	8	66
Total	107	11	111	36	265

produced twenty-eight normal to five defective offspring, which is significantly lower than an expected 1 to 1 ratio. Furthermore the mating of defective ewes with the three defective rams gives ratios of defective to normal offspring which are much too low to conform with the hypothesis that the overshot condition is due to simple recessive inheritance. Misclassification of some animals due to lack of complete penetrance or environmental variation may have caused some of the discrepancies which prevent any simple genetic interpretation of the data in table 6. In this connection, however, it should be remembered that the normal and defective daughters were from defective ewes and from the two defective rams, 3408R and 3872R. Consequently they should have been genetically alike if the defect were recessive. A X^2 test of the 28 to 5 and the 10 to 12 ratios observed for normal and defective daughters ($P < .002$) from the normal dams and defective dams, respectively, indicates that the two groups were genetically different, the probability of obtaining defective offspring from the defective groups being significantly greater than for the normal group. Additional evidence in this

connection is that the three groups of defective dams were genetically different, the probability of obtaining defective offspring decreasing with the relationship of sire and dam.

The events which led to the use of 2560W in these matings are of further interest. The sire of 2560W was a ram of outside breeding purchased to initiate an inbred line. All of his offspring had normal jaws until the third year when he was mated to his first daughters. Three of nine lambs from these daughters had badly overshot jaws while all offspring from unrelated ewes were normal. The overshot lambs were small and weighed an average of 34 pounds less at weaning time than the other six inbred lambs. The following year a normal son (2560W) was used in the line. He was bred to a number of half-sisters and two overshot lambs out of thirteen were produced. These overshot lambs were also small. A second change of sires (half brother to 2560W) was made and no defective offspring have been born in this line during the succeeding 3 years.

Again it appeared from these results that 2560W and his sire were each heterozygous for a recessive gene causing the overshot condition. The observed ratio of seventeen normal and five defective was not significantly different from an expected 7 to 1 ratio. Then 2560W was mated to normal daughters of 3408R and 3872R and to other overshot ewes to determine if the genetic basis for the overshot condition in daughters of 2560W was independent of those operating in the other matings. Two defective lambs out of twenty-three were produced by mating 2560W to normal daughters of 3408R and 3872R. This is similar to the ratio obtained by mating defective rams to normal ewes and is significantly different from an expected 3 to 1 ratio. One defective lamb out of sixteen was produced when 2560W was mated to defective ewes. This is also significantly different from an expected 1 to 1 ratio. Therefore, it appears possible that the defect appearing in inbred offspring of 2560W and his sire may be identical to that found in the regular overshot matings which appeared at first to be simple recessive and later proved to be more complicated. It is also possible that the defect coming from 2560W and his sire may actually be a simple recessive trait which is inherited independently of the defect found in the regular overshot matings. The dwarf size of the defective inbred offspring of 2560W and his sire furnished some evidence that this condition is distinct from the defect found in some offspring of 3408R and 3872R. Matings with 2560W and his offspring were not carried far enough to prove or disprove these possibilities. Bogart and Dyer ('42) reported on overshot condition in Southdown sheep associated with dwarfism which appeared to be a simple recessive lethal.

Mode of inheritance

In the early phases of the investigation several facts indicated that the overshot condition was due to simple recessive inheritance: (1) The defect segregated from normal matings with low frequency (1.4% among nearly 7,000 Rambouillet lambs); (2) two matings of normal rams to their normal daughters gave normal and overshot offspring suggestive of monohybrid ratios, and (3) one son of a ram presumably heterozygous for the defect gave a ratio corresponding to seven normal to one overshot offspring when mated to his half-sisters, whereas a half-brother mated to the same ewes produced no defective offspring. Kelley ('42) observed similar segregation from mating a ram to his daughters and postulated that the condition was due to recessive inheritance. However, this hypothesis was refuted when the first experimental matings of defective rams and defective ewes produced so few defective offspring and became increasingly untenable as additional data were obtained.

Supplementary hypotheses involving incomplete penetrance or non-hereditary variation, leading to misclassification of parents or offspring, are inadequate to explain the discrepancies to the hypothesis of simple recessive inheritance because: First, the frequency of the defect from matings of defective parents was found to depend upon the closeness of the mating, whereas misclassification presumably would be independent of the type of mating. Second, normal and defective daughters from defective ewes, when mated to their sires, would be expected to produce defective offspring with the same frequency. The observed ratios were twenty-eight normal to five defective as compared to ten normal to twelve defective.

These circumstances, showing that both pedigree and phenotype of the ewes are independently associated with the frequency of the defect in the offspring, appear to exclude any simple genetic interpretation of the results. The low frequency of defective offspring from unrelated defective parents as compared with the higher frequency from related defective parents suggests that several genes are involved in producing the defect. The frequency of the genes responsible for the defect was obviously increased by inbreeding to the defective rams during the course of the experiment.

The nature of these genes cannot be fully determined from this study. The evidence appears conclusive in eliminating one or more pairs of recessive genes as the sole cause of the defect. Some of the genes are most certainly dominant and additional recessive genes could be in-

volved. It seems most plausible that the defect results from interactions among several pairs of genes.

SUMMARY

A study of inequalities of the jaws of sheep has been conducted over a period of more than 7 years at the Western Sheep Breeding Laboratory and United States Sheep Experiment Station, Dubois, Idaho. Illustrations of instruments are presented which have been developed to measure angles of various skull structures in order to clarify the etiology of the defects.

Sheep with overshot jaws had longer skulls and shorter mandibles than normal animals. The mandible was affected along its entire length, but the space occupied by the molar teeth was less affected than the remainder of the jaw. The maxilla was the only structure of the upper jaw that was appreciably longer in overshot ewes than in normal ewes and again it was affected much more than the molar space.

Linear skull measurements were more variable in overshot than in normal animals, reflecting greater general disharmony in skeletal structures of defective animals. However, positive correlations were found between upper and lower jaw structures in both overshot and normal groups indicating that general factors are important in determining variation in different skeletal structures despite the disharmony evinced by the overshot condition. There is no conclusive evidence that the angle of the mandible was disturbed by the overshot condition.

A tendency was apparent in normal animals for the angles of the incisor teeth to compensate for inequalities of the jaws. These angles in normal sheep were inversely correlated with mandible length and were much more variable in normal than in overshot animals. This tendency to compensate was absent in the overshot animals studied.

Marked inequality of the upper and lower jaw occurred in about 1.4% of the offspring from a large flock of normal Rambouillet sheep. Experimental matings in which one or both parents were overshot produced 16.4% defective offspring. Defective lambs were slightly smaller than normal but no reduction in viability to weaning age was apparent. Inequality of the jaws was found to increase from birth to weaning to yearling age.

The occurrence of the overshot condition from matings of normal parents suggested a simple recessive inheritance of the defect. However, this hypothesis was refuted when the mating of defective rams with defective ewes gave too few defective offspring. When defective rams were mated with normal and overshot daughters, a significantly

greater proportion of defective offspring were obtained from the overshoot daughters indicating that they were genetically different from the normal daughters. Furthermore, three groups of defective dams were found to be genetically different, since the probability of obtaining defective offspring decreased with the relationship of sire and dam. This indicates that interactions of several pairs of genes were probably involved. Some of these genes were most certainly dominant and additional recessive genes could have been involved.

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EFFECTS OF ANOXIA UPON CAROTID BODY MORPHOLOGY ¹

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ONE PLATE (TWO FIGURES)

INTRODUCTION

There is general anatomical as well as physiological and pharmacological evidence that chemoreceptor cells actively participate in the initiation of the chemoreceptor reflexes. However, cytological changes which might be presumed to occur upon activity of these cells have apparently never been investigated.

Studies of the carotid body have indicated that a prominent feature of the specific cells forming this organ is their granulation, best demonstrated by mitochondrial techniques. De Castro ('26) described these granules as consisting of two types, true mitochondria and lipoid granules. The latter he believed to be a secretory or pre-secretory product of the cells, which at that time he interpreted as forming an endocrine organ. In a later study ('28) he discovered the sensory nature of the carotid body, but offered no further interpretation of these granules. The present author (Hollinshead, '43) has also, however, adduced evidence that the majority of the granules are probably not mitochondria, and has suggested that they play some part in the initiation of carotid body reflexes.

In the present study, the effects of prolonged anoxia upon the cytology of the carotid body cells, especially in regard to the granules, have been investigated. The study has indicated that such stimulation may result in a marked depletion of the granules.

MATERIAL AND METHODS

Mice were used in this study. The experimental animals, divisible into two series, were subjected to various degrees of anoxia in a gas chamber for periods of 2 or more hours. In the first series, comprising twenty-three animals, the oxygen level in the gas chamber was maintained at $10 \pm 1\%$ throughout the course of the experiment; at the end of 2, 4, 6, or 8 hours the animal was killed by breaking its neck.

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In the second series, of eleven animals, the mice were placed in the gas chamber at an oxygen concentration of about 10%, and allowed to remain there without the addition of further oxygen until death from anoxia occurred. As a rule, this took place in approximately 4 to 6 hours, as the O_2 level in the chamber fell to between 6 and 5%. All animals in both series showed immediate and continued respiratory stimulation, marked by greatly increased depth of respiration, upon being placed in the gas chamber. They usually soon became lethargic except for these forced respiratory movements.

The gas chamber was a small-mouthed 5-gallon glass bottle with a rubber stopper. Two outlets were connected as needed to a vacuum pump, a mercury manometer, a cylinder of nitrogen, a flask filled with oxygen, and a Haldane gas analysis apparatus. Soda lime was suspended in the chamber to absorb the CO_2 .

In running an experiment, about half the air was withdrawn from the chamber, as indicated by the manometer. Nitrogen was then introduced to restore normal atmospheric pressure. After mixing the gases, a mouse was introduced into the chamber. This was then resealed, and the composition of its contained air was determined by gas analysis. In those cases in which the O_2 level was kept relatively constant, oxygen was fed into the chamber from another container by the drip method. The maintenance of the O_2 level in the first series, and its decrease in the second series, were substantiated by gas analyses repeated at 1- or 2-hour intervals throughout the duration of each experiment. In no case was CO_2 concentration found to have reached a measurable level.

The carotid bifurcations of the experimental animals, and of normal animals killed by breaking their necks, were fixed in Regaud's fluid. The tissue was sectioned serially at 6μ ; that from one side of the animal was stained by the Bensley-Cowdry method for mitochondria, that from the other by the Severinghaus technique for the hypophysis.

OBSERVATIONS

The carotid body of the mouse is usually closely attached to, and sometimes imbedded within, the superior cervical sympathetic ganglion. In general it resembles that of the cat, being a fairly compact body of great vascularity. As in the cat, the cells present a faintly granular appearance with the Masson technique, and with some general cytoplasmic stains, but the granules which the cells contain are brought out clearly only by mitochondrial techniques. Cell outlines are fre-

quently difficult to decipher in this animal, but in general the cells are round to oval.

The specific cells forming the carotid body show, in normal mice, considerable variation in regard to the density of the granules; the cytoplasm of some cells is almost completely filled with closely packed granules, while in others the granules are more scattered. In addition to these cells, and to mast cells which are also recognizable with the Bensley-Cowdry technique, there are usually a few small stellate cells, whose cytoplasm is crowded with granules which stain brilliantly with fuchsin, wedged in among the larger, more rounded chemoreceptor cells. I have been unable to determine the nature or significance of these smaller cells; they have not been seen in all mice, but occur sparingly in both normal and experimental tissue. The nature of their granulation is also unknown.

The carotid bodies from the twenty-three animals subjected to a maintained level of anoxia for 2 to 8 hours showed in general no clear cut deviation from the normal pattern. Among the nine cases in which the anoxia lasted for 2 or 4 hours there were two in which the distribution of granules appeared somewhat more dense, on the whole, than in any of eight normal animals; also, among the fourteen in which the anoxia was maintained for 6 or 8 hours, there were eight in which there seemed to be some depletion of granules. Neither of these apparent changes were of such proportions as to be convincing, however; therefore, the results of this entire series are equivocal.

On the other hand, the changes were marked and consistent in the carotid bodies from the eleven animals in the second series, in which the oxygen concentration was allowed to decrease slowly to a lethal level. In every case there was a decrease in the average granulation of the cells. In two of these cases, there was marked degranulation of only a few cell groups, and moderate degranulation of others, while some cells were still well filled with granules; in four others, the majority of the cell groups were greatly degranulated, and in the remaining four practically every cell was degranulated. In its extreme, this degranulation practically emptied the cytoplasm (fig. 2). With less degranulation, the remaining granules were sometimes scattered apparently at random; not infrequently, however, the majority were situated in the periphery of the cell, forming in some cases a clear cut peripheral ring. The small stellate cells appeared, however, to be unchanged either in number or granulation.

Carotid bodies from five animals subjected to 40% oxygen for periods of 2 and 4 hours were also examined, but showed no detectable changes from the normal.

The superior cervical ganglia of the normal and experimental animals were also compared to determine the effect of anoxemia on this organ. However, the experiments reported here had induced no observable changes in the mitochondria of the nerve cells.

DISCUSSION

The changes in granulation seen in the carotid body cells of animals subjected to prolonged and severe oxygen lack indicate strongly that anoxia may affect the morphology of chemoreceptor cells. These changes have occurred, however, only upon extreme anoxia; less severe oxygen lack, even though extended over a period of 8 hours, has produced no definite and consistent change. Since the oxygen level used in these experiments has in all cases been accompanied by increased respiratory activity, the question arises as to whether the degranulation must be attributed to anoxia, *per se*, of the carotid body cells, or whether it is not rather an indication of excessive activity of those cells.

The effect of oxygen deprivation upon most tissues is perhaps to a slight extent debatable, but primarily it results in a depression of cellular activity. Exactly the reverse is true of chemoreceptor tissue, for anoxemia is a specific stimulus for initiation of activity on the part of this tissue. The apparent rôle which the specific cells play in the initiation of these reflexes has recently been discussed by Hollinshead and Sawyer ('45); it suffices to say here that there is good evidence that the chemoreceptor cells themselves are responsible for the initiation of the reflexes, and therefore become more active, at least within limits, as the oxygen tension of their milieu decreases. The variation in granulation of the normal carotid body cells is in itself suggestive that these granules are concerned with activity of the cells. Their depletion in severe anoxia is most marked in those animals in which oxygen lack led to respiratory failure and death, and it may indeed be a primary contributing factor to this latter.

Anoxemia in itself has no apparent effect upon the dying or dead cells of chemoreceptor tissue, for if fixation is delayed even for as long as 2 or 3 hours after death, in a normal animal, the carotid body cells show no appreciable change in granulation from that of cells fixed immediately after death, in spite of the severe and prolonged anoxemia to which they have been subjected. The degranulation observed in the experimental material seems therefore to be an active process, associ-

ated with some phase of cellular activity. One phase of activity, namely the specific chemoreceptor function, is known to be stimulated by anoxemia; in the carotid body a decrease in the granulation of the cells seems therefore to be correlated with extreme and probably prolonged stimulation of this tissue. It thus appears that the granules within the carotid body cells probably play a specific part in the initiation of chemoreceptor reflexes.

The present experiments can obviously not be interpreted as elucidating the nature of these granules. In a previous paper (Hollinshead, '43), evidence was presented that they are not mitochondria although there are probably some mitochondria among them. Unfortunately it is difficult and usually impossible to distinguish between the apparent mitochondrial and non-mitochondrial granules in fixed preparations. It is quite possible that most of the granules remaining in otherwise degranulated cells represent mitochondria, and that the non-mitochondrial granules are in fact almost completely exhausted. The mitochondria of the nerve cells have apparently not been affected in the present experiments, judging by the condition of the superior cervical ganglia; whether this also holds true for the carotid body is unknown. Insofar as the carotid body is concerned, no previous experimental work of the present nature has been reported, to this author's knowledge. De Castro ('26) described an increase in granulation of the cells of the cat carotid body following section of the innervation of this organ, and interpreted this as a further indication of its endocrine nature. How this finding can be related to our present concepts of chemoreceptor function is not clear.

At the beginning of the present study, it was anticipated that the changes induced in the carotid body might be of such magnitude that a cytological method could be used to confirm the physiological and pharmacological evidence indicating that chemoreceptor stimuli can be divided into two great groups: those affecting the chemoreceptor cell, and those affecting the sensory pathway central to the cell. As changes of sufficient magnitude have occurred only upon prolonged and severe stimulation, which would be difficult to repeat with drugs, pharmacological evidence such as that presented by von Euler, Liljestrand and Zotterman ('39) must remain the method of choice for investigating this question.

The absence of obvious change in the granulation of the carotid body cells of many animals subjected to a rather severe, but not lethal, anoxia, is probably to be correlated with the difficulty of fatiguing the chemoreceptor reflexes. Whatever the exact role of these granules, it

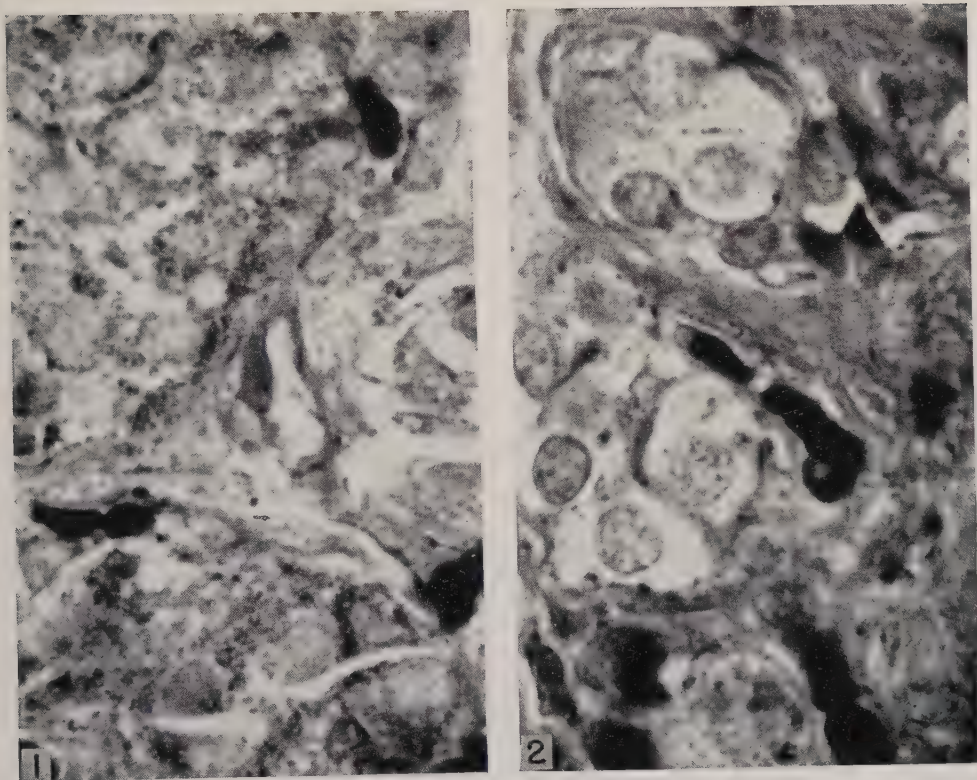
is reasonable to believe that they are intimately concerned with chemoreceptor function; and they are apparently maintained in approximately normal numbers even during prolonged activity of the cells. The variations in granulation in different cells, and the apparent slight increase in granulation observed in some cases after short periods of stimulation, suggest that new granules are probably constantly being formed as others are exhausted. While there are variations in the sizes of the granules, it has not been possible to correlate these with the degree of stimulation of the cells, or the amount of degranulation present.

SUMMARY

Effects of anoxia on the morphology of the carotid body were tested in two series of mice. In the first series, in which the oxygen level was maintained at $10 \pm 1\%$, no marked or consistent changes were observed, although there was clear physiological evidence of stimulation of the chemoreceptors. In the second series, in which the oxygen was gradually reduced to a lethal level, pronounced degranulation of the carotid body cells was observed. This degranulation is believed to be correlated with excessive activity of the chemoreceptor cells, and is therefore interpreted as evidence that the granules in these cells are directly concerned with initiation of chemoreceptor reflexes.

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Unretouched photomicrographs of portions of sections of carotid bodies of mice. Stained by the Bensley-Cowdry technique. $\times 1700$, as reproduced.

1 Carotid body of a normal mouse. The definitive carotid body cells occur in groups, and the cytoplasm of these cells is for the most part rather heavily loaded with granules. While the granulation varies somewhat between various normal carotid bodies, and between cell groups in the same specimen, the cells shown here present an approximate average granulation for normal material.

2 Carotid body from a mouse stimulated by anoxia for 5 hours and 10 minutes. During this time the oxygen level dropped from 10.4% to 5.2%; the latter was a lethal level for this animal. The carotid body cells from this animal, as illustrated here, were almost completely degranulated, only an occasional granule being visible in the cytoplasm.

A STUDY OF THE ESTROUS CYCLE IN THE GOLDEN HAMSTER, *CRICETUS (MESOCRICETUS) AURATUS* WATERHOUSE¹

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ONE PLATE (SIX FIGURES)

INTRODUCTION

The estrous cycles of laboratory born and bred specimens of *Cricetus auratus* have been studied by the vaginal smear technique. The duration of the cycle and of its phases has been investigated, and the cyclic changes in appearance of vaginal smears have been described in detail for the first time. Deanesly ('38) first studied the reproductive cycle in the golden hamster as exhibited by the ovary and secondary sex organs and observed that the cycle occupies 4 days. Peczenik ('42), Kupperman, Greenblatt, and Hair ('44) and Sheehan and Bruner ('45) have made additional observations relative to the estrous cycle and reproduction in this form. Chang and Wu ('38) in studying the growth and reproduction of *Cricetus griseus*, the striped, or Chinese hamster, found that the feral animals in their natural habitat exhibit irregular cycles which remain irregular when they are confined in the laboratory, but that laboratory born and bred siblings exhibit a normal estrous cycle the duration of which averages $4\frac{1}{2}$ days. No further details are given concerning the estrous cycle of the latter species. Parkes ('31) studied the estrous cycles of each of five normal specimens of *Cricetulus griseus* and found an average duration of 4.3 days for the sixty-nine cycles observed.

MATERIALS AND METHODS

The data herein contained are based on 184 complete cycles exhibited by twelve laboratory born and bred, sexually mature, virgin females. The cycles were observed over a period of 8 months and represent both consecutive and non-consecutive series. One hundred and fifty-nine complete cycles were observed at 24-hour intervals; twenty-five addi-

¹ Contribution no. 72 from the Department of Zoology, Physiology, and Entomology, Louisiana State University.

tional complete cycles were observed at 8-hour intervals, 7 A. M., 3 P. M., and 11 P. M. From the data presented one will note that, where relatively large numbers of observations are made at 24-hour intervals, values indicating the duration of the cycle are obtained which vary only slightly from values obtained when observations are made every 8 hours. The 8-hour procedure is essential, however, for determining the duration of a single phase of the complete cycle.

Vaginal smears were made in a drop of physiological saline from a platinum loop and were studied unstained. Staining was found to be unnecessary as the details of the preparation were readily observed by this method, and the cellular contents of the vagina were easily identified. The accompanying figures were drawn from fresh unstained smears and represent characteristic stages in the estrous cycle of the hamster.

The authors are indebted to Mr. Victor Schwentker of Tumblebrook Farm, Brant Lake, New York, who generously provided the original stock from which the experimental animals were bred.

OBSERVATIONS

The average duration of the estrous cycles of each of twelve hamsters, as determined from smears made once daily, is indicated in table 1. The average duration of the cycle for all twelve animals may be calculated employing the figures from column two of the table, and this

TABLE 1

The average duration of estrous cycles among 12 hamsters as determined from daily smears during 159 total cycles; and among 10 animals as determined from smears made three times daily during an additional total of 25 cycles.

ANIMAL NUMBER	AVERAGE DURATION IN DAYS BASED ON DAILY SMEARS	NUMBER OF CYCLES OBSERVED	AVERAGE DURATION IN HOURS BASED ON 8-HOUR SMEARS	NUMBER OF CYCLES OBSERVED
1	3.992	22	100	2
2	4.000	14	100	2
3	4.045	11	104	2
4	4.045	11	104	2
5	4.066	15	88	3
6	3.964	14	100	2
7	4.078	19	88	3
8	4.033	15	96	2
9	3.642	14	74	4
10	3.916	18	90.6	3
58	4.166	3		
61	4.000	3		

average cycle will be found to occupy 3.9 days (or 95.9 hours). The average cycle for ten of these animals may similarly be calculated, employing the figures from column four of the table, and may be computed to occupy 94.4 hours (or 3.9 days). One must thus conclude that, among the specimens of *Cricetus auratus* used in the present investigation, the estrous cycle occupies an average of 3.9 days. This finding is in keeping with our casual observation that, for practical purposes, the hamsters in our colony may be found to come into heat every fourth day, or on days 1, 5, 9, 13, et cetera.

Vaginal contents during the estrous cycle

Consideration of the vaginal smear picture of the golden hamster at any one time concerns the relative numbers of three cell types: squamous cells (nucleated or non-nucleated); smaller, nucleated, cuboidal cells; and leucocytes. The appearance and relative density of each of these cell types first will be considered independently of the other cell types throughout a typical cycle. The complete smear picture at representative phases of the cycle will then be described.

Squamous cells. These cells appear in large or small numbers throughout the entire cycle. When squamous cells represent nearly the sole cell type appearing in the vaginal smear the animal may be considered to be in estrus, for reasons which will be discussed later. During estrus the cells are large, often polyhedral, and the edges usually are not frayed nor are the cells fragmented. Many of the cells are cornified and nuclei are not visible in the fresh smear (fig. 2). The precise number of squamous cells appearing in a given field varies with the number of hours the animal has been in estrus, the mid-estrus period exhibiting the smallest number of cells. The density of such cells in the smear during estrus may be characterized as "sparse." This sparse phase terminates with the influx, slow at first, then rapid, of great numbers of spindle-shaped, or elongated squamous cells many of which are aggregated into fascicles representing masses of desquamated tissue. Approximately 8 hours after the first appearance of elongated squamous cells the smear picture has changed completely, and the smear now consists predominantly of enormous numbers of elongated squamous cells which completely fill all fields (fig. 3). Such a distribution of cells is referred to as "abundant." Approximately 8 hours after the abundant elongated stage the picture has again changed completely. Squamous cells have decreased in number and few elongated cells remain. At this time the squamous cells may be masked by other cell types. The squamous cells now, and thereafter for many hours,

are large or small with regular or serrated borders. Few or no cornified cells appear. Approximately 36 hours after the initial influx of elongated squamous cells the number of squamous cells of all types falls off to sparse at which level they remain until the advent of the next estrous period.

Cuboidal cells. Large or small epithelial cells circular or oval in outline with relatively large, prominent nuclei are herein classified as cuboidal cells. They lie deeper within the vaginal epithelium than do the squamous cells. Cuboidal cells are typically absent during estrus and do not appear in numbers in vaginal smears until the influx of elongated squamous cells is well advanced. They are present in small numbers at the peak of the abundant elongated squamous cell phase (fig. 3). When this peak has been reached by the elongated cells cuboidal cells invade the smear in rapidly increasing numbers and within 12 hours these cells have attained their maximum concentration. This condition is maintained 8 to 16 hours. At this stage the numerical distribution of cuboidal cells may be characterized as "abundant" (fig. 4). They appear in large numbers in every field, but not to the exclusion of other cell types nor to the extent of masking other cell types. Following this phase in the cuboidal cell cycle the cuboidal cells decrease rapidly in number falling, within the ensuing 16 to 24 hours, to sparse where they remain until some time prior to heat when they progressively decrease in number once more until they have disappeared almost altogether from the smear at the onset of the heat picture described in the previous paragraph.

Leucocytes. Leucocytes are typically absent from the smear during estrus, and almost absent during the period of influx of elongated cells following estrus. They commence to make their appearance in the vaginal lumen in numbers approximately 4 hours or more after the cuboidal cells have commenced their influx, and the number rapidly rises within the course of 8 to 16 hours until they have attained a condition of abundant, and are as numerous as, or more numerous than, the cuboidal cells at the end of this time (fig. 4). From this abundant condition the leucocytes slowly fall off in number, along with the cuboidal cells, or slightly behind them, within the next 16 to 24 hours, to sparse (fig. 6). Here they remain until just prior to heat when they progressively decrease in number until they have disappeared altogether from the smear at the onset of the heat picture previously described.

Estrus. As already indicated, when the smear picture is characterized by the presence of squamous cells alone the animal is considered to be in estrus (fig. 2). Many of the squamous cells are cornified, and

they occur sparsely throughout the smear. In early estrus the cells are more numerous and a few are fragmented. A few cuboidal cells and perhaps a single leucocyte may also be present in some of the fields. By mid-estrus and late estrus the fragments and cuboidal cells have disappeared from the smear and the squamous cells have decreased in number, with cornified cells predominating. The mid-estrus smear is typically diffuse. The region of the vulva is dry, and little fluid occurs within the vaginal lumen.

Metestrus. Estrus is considered as terminating with the influx, slow at first, then rapid, of large numbers of elongated squamous cells. Eight hours after their first appearance the elongated squamous cells constitute almost the sole cell type present. By this time the smear is dense, and consists predominantly of very large numbers of elongated squamous cells which more or less completely fill all fields (fig. 3). This phase of the cycle is accompanied by a relatively thin, cream-colored secretion occurring during the early stages high in the vaginal cavity, later filling the lumen of the vagina throughout its entire extent. The peak elongated squamous picture is of short duration (4 to 8 hours) and hence is often overlooked unless smears are made at 4-hour intervals.

Diestrus. At the peak of the elongated squamous stage cuboidal cells commence to appear in the lumen, followed shortly by leucocytes. As cuboidal cells and leucocytes increase in number the elongated squamous cells disappear rapidly so that, 8 hours after the peak of the elongated squamous stage, an entirely different and characteristic stage is observed in which leucocytes and cuboidal cells are present in abundance, everywhere filling the field, and producing what we refer to as a "leucocyte-cuboid" picture (fig. 4). At this time the cream-colored secretion is copious, and readily oozes out of the genital aperture to cover the vulva. The secretion is accompanied by a lingering, repugnant odor which is diagnostic of this phase of the estrous cycle. In the event the fluid does not spontaneously ooze from the vagina slight pressure in the pelvic area will reveal its presence. This leucocyte-cuboid picture may be expected to persist about 12 hours, and is the typical picture which may be observed during day three of the cycle (which begins with the onset of estrus) or approximately 48 hours after mid-estrus. Squamous cells occur but are masked by the large number of leucocytes and cuboidal cells. The leucocyte-cuboid picture becomes modified by an abatement of the influx of cuboidal cells so that, following the peak of the leucocyte-cuboid picture, the leucocyte emerges as the predominating cell type present (fig. 5). By the sixteenth to twentieth hour after the termination of the typical leucocyte-cuboid picture, both leu-

cocytes and cuboidal cells have decreased to a sparse distribution, where they remain, along with squamous cells (fig. 6). This typical sparse picture obtains for approximately 24 hours and represents the typical picture encountered on the fourth day of the cycle. At this time squamous cells, leucocytes, and cuboidal cells are present in moderate or small numbers and the smear tends to be diffuse. Many of the cuboidal cells are larger than in the earlier smears and apparently are undergoing transition to the squamous state.

Proestrus. Toward the end of this sparse period which terminates diestrus, leucocytes and cuboidal cells simultaneously disappear rapidly from the smears. Squamous cells now predominate and are sparse; cuboidal cells appear to the extent of a few in each field; leucocytes have reached a minimum number, averaging one or two to each high power field (fig. 1). At this time the vulva is dry or slightly moistened with a watery secretion. This phase we have termed proestrus. It may be considered to terminate when leucocytes have disappeared completely from the smear. The absence of leucocytes and presence of squamous cells indicates that the animal has reentered estrus.

The duration of estrus

The foremost criterion for determining the state known as "heat" in the female is the responsiveness of the female to the advances of the male, as evidenced by the exhibition, sooner or later, of lordosis. The true meaning of the word estrus is thus emphasized as referring to the time when the female exhibits sexual desire. This phenomenon of heat is a manifestation of a total physiological and psychological state obtaining within the organism and is correlated with, but need not always be exactly synchronous with, a single vaginal smear picture. Mixner and Kent ('45) have shown that a high per cent of successful matings will occur if the male is placed with the female when the latter is in estrus as herein defined. Later experiments by the same authors, not yet released, will show that matings made at all other times (with the exception of metestrus) typically prove unproductive. Deanesly pointed out that a sticky discharge, rich in epithelium, may be squeezed from the vulva every 4 days, and demonstrated that this discharge was associated with a ruptured follicle stage. It was correctly referred to as a "post-oestrus" discharge. Peczenik observed that a mating response obtains during this interval between the cornified stage and the leucocyte stage. He therefore concluded that the period of discharge was the period of heat and suggested further that the rich leucocyte

smear immediately following the epithelial discharge indicated, in all probability, metestrus. All our observations indicate that lordosis may be elicited in proestrus as herein defined, that receptivity in the female increases as estrus is entered, and that it continues throughout estrus and into the short period of metestrus (Deanesly's "post-estrous discharge" period), then ceases. It is probable that the egg is released from the ovary in the final stages of estrus. This would explain the fact that successful matings still occur in metestrus, and is in harmony with Deanesly's and Peczenik's observations that, at this time, the ovaries already contain fresh corpora lutea. Sheehan and Bruner correctly consider the stage of relatively few cornified cells to be estrus.

TABLE 2

Table showing the average duration of estrus in hours in each of ten hamsters observed at 8-hour intervals.

ANIMAL NUMBER	NO. OF CYCLES OBSERVED	AVERAGE DURATION OF ESTRUS IN HOURS
1	2	24
2	2	24
3	2	26.6
4	2	29.3
5	3	18.6
6	2	26.6
7	3	26.0
8	2	32
9	4	30
10	3	37.3

In the present paper the animal is considered as entering estrus when leucocytes disappear from the vaginal smear. Estrus pictures may be observed in which a single leucocyte or a very few leucocytes occur in the entire smear, but typically field after field may be observed in which leucocytes are entirely lacking. Estrus is defined as terminating with the influx of elongated squamous cells. Delimiting estrus by these criteria the approximate duration of this phase of the cycle in ten animals has been calculated from observations made at 8-hour intervals. Table 2 summarizes these data. For all ten hamsters estrus exhibits an average duration of 27.4 hours.

SUMMARY

1. The average duration of the estrous cycles among twelve specimens of *Cricetus* (*Mesocricetus*) *auratus* Waterhouse as determined

from vaginal smears observed during 184 complete cycles has been found to be 3.9 days.

2. The vaginal contents during estrus, metestrus, diestrus, and proestrus have been described.

3. The period of estrus, during which few or no leucocytes appear in the vaginal lumen, and when squamous cells are the typical cell type present, has been found to occupy 27.4 hours.

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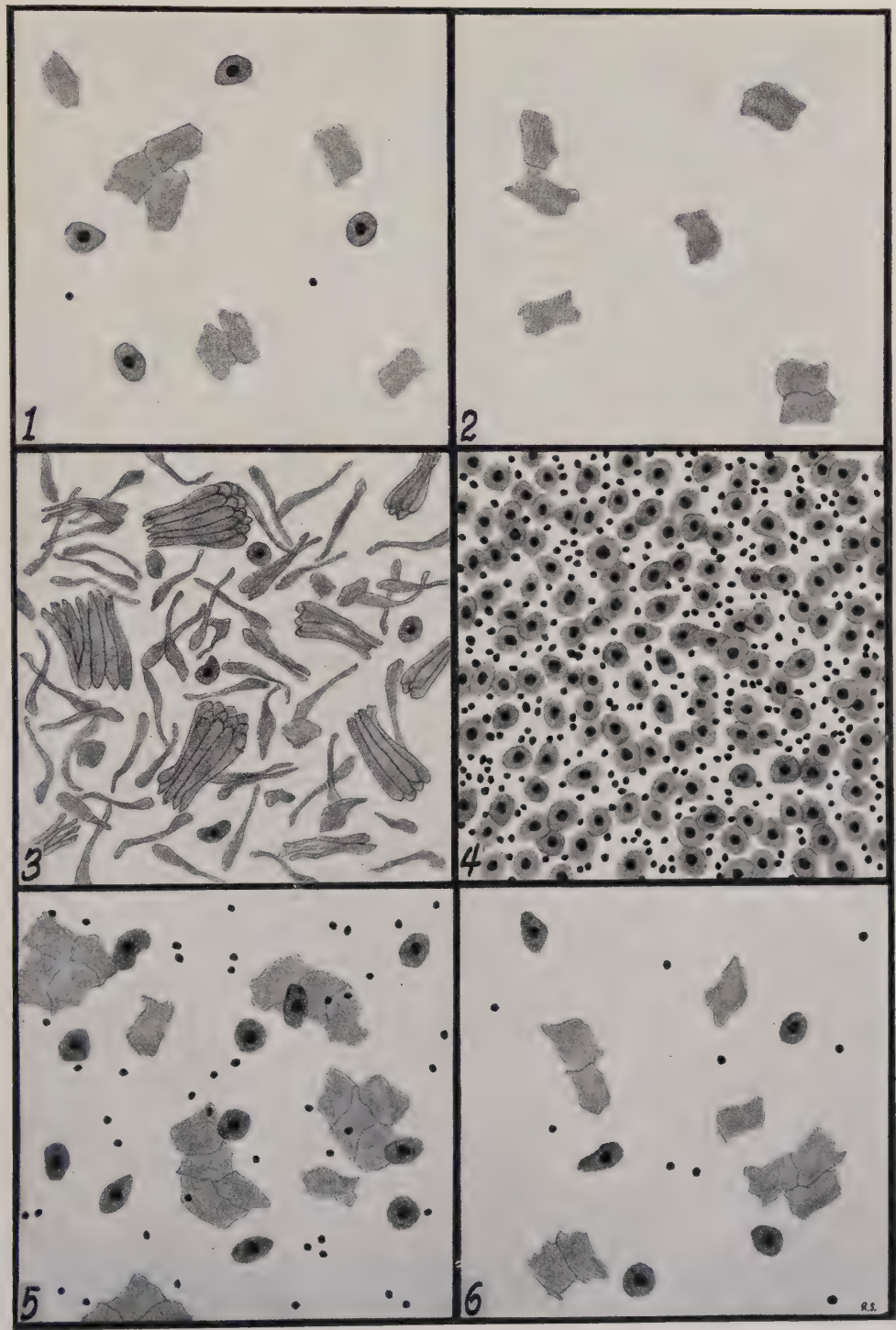
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PLATE 1

EXPLANATION OF FIGURES

Vaginal smears of the golden hamster, *Cricetus (mesocricetus) auratus* Waterhouse, showing the characteristic cell types and their relative abundance during the estrous cycle. $\times 200$.

1. Proestrus. The smear contains scattered squamous cells, a few cuboidal cells, and one or two leucocytes.
2. Mid-estrus. The smear consists of a few scattered squamous cells.
3. Metestrus. The smear shows an abundance of elongated squamous cells with a few cuboidal cells.
4. Diestrus (early). The smear consists of cuboidal cells and leucocytes in abundance. Squamous cells occur in considerable numbers but have been omitted in the drawing since they are masked by the other cells.
5. Mid-diestrus. The smear contains leucocytes as the predominating cell type with scattered cuboidal and squamous cells.
6. Diestrus (late). The smear consists of scattered leucocytes, cuboidal cells and squamous cells.



THE LARYNGEAL REGION OF ALLIGATOR MISSISSIPPIENSIS

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FOUR FIGURES

It is, of course, well known that the alligator's throat is arranged to allow the alligator to hold a struggling animal under water without permitting water to enter the alligator's trachea; this arrangement also permits the alligator to come to the surface to breathe while still holding its prey in its mouth.

Figure 1 shows plainly this apparatus consists of a ventrally-projecting, transverse, palatal fold (p) and a dorsally-projecting lingual fold (l). The nasal chamber, above the palatal bone, opens behind these folds and permits free passage of air from the anterior nares to the glottis.

It is possible that the virtually single palatal fold may have been derived from the palato-glossal folds, the forerunners of the pillars of the fauces of higher forms. From the relative positions of these structures this seems likely.

The snug way in which these two folds fit together is shown in figure 2, a sagittal section of the head of an alligator embryo taken just before hatching. The section shows the general shape of the nasal cavity (n) opening at the anterior naris (a) and leading back to the glottis (g). A sagittal section of the large basilingual plate of the hyoid is shown at b; a sagittal section of the cricoid plate of the larynx is shown at cr; and one of the small arytenoid cartilages is shown at ar. The glottis (g) seems very wide because it is cut through its longitudinal diameter. The trachea (t) and the esophagus (e) have the usual appearance, and in the former several of the approximately fifty tracheal rings found in the alligator may be seen at tc.

The large palatal and the lingual folds (p and l) show plainly how the anterior and posterior oral regions can be separated.

THE HYOID APPARATUS

The hyoid, which has been briefly described by the writer in another place (Reese, '15), is a large, chiefly cartilaginous structure that covers

most of the ventral surface of the throat at the base of the tongue. It consists of three parts: the large, median basilingual plate or body and the lateral cornua (fig. 3).

The basilingual plate (b) is very large in proportion to the size of the animal; it is very thin and is composed entirely of cartilage. It is depressed medially where it underlies the ventral curvature of the

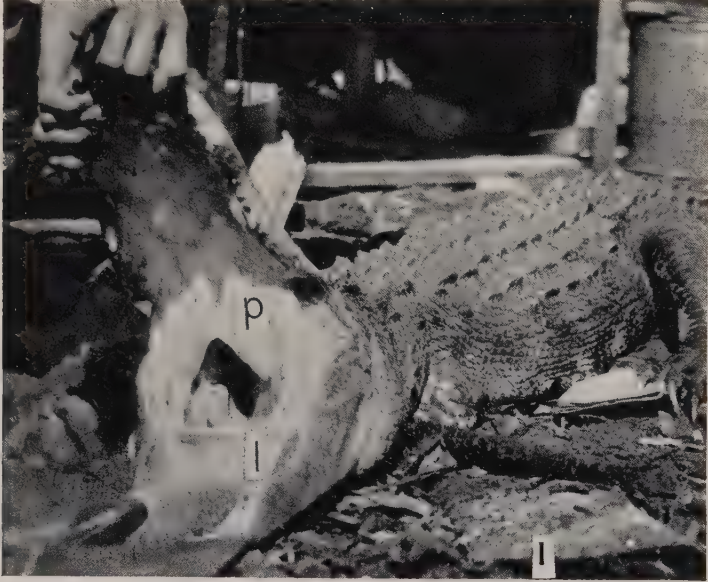


Fig. 1 Open mouth of *Alligator mississippiensis* to show the lingual folds, l, and the palatal fold, p.

ABBREVIATIONS

a, anterior naris	g, glottis
ar, arytenoid cartilage	l, lingual fold
b, basilingual plate	n, nasal cavity
e, anterior cornu	p, palatal fold
er, ericoid cartilage	t, trachea
e, esophagus	tc, tracheal cartilage
f, false vocal cords	tn, tongue

throat. The anterior margin is a wide, almost circular curve, with the convexity of the curve towards the head. The posterior margin is narrower, with a wide notch, opening caudad. The lateral borders of the basal plate are notched, and from each notch extends latero-caudad a long cornu (c). Each of the cornua is a long, narrow structure, narrowest at its base and gradually widening towards its caudal end. It is ossified throughout most of its length, with a flat caudal extremity of cartilage.

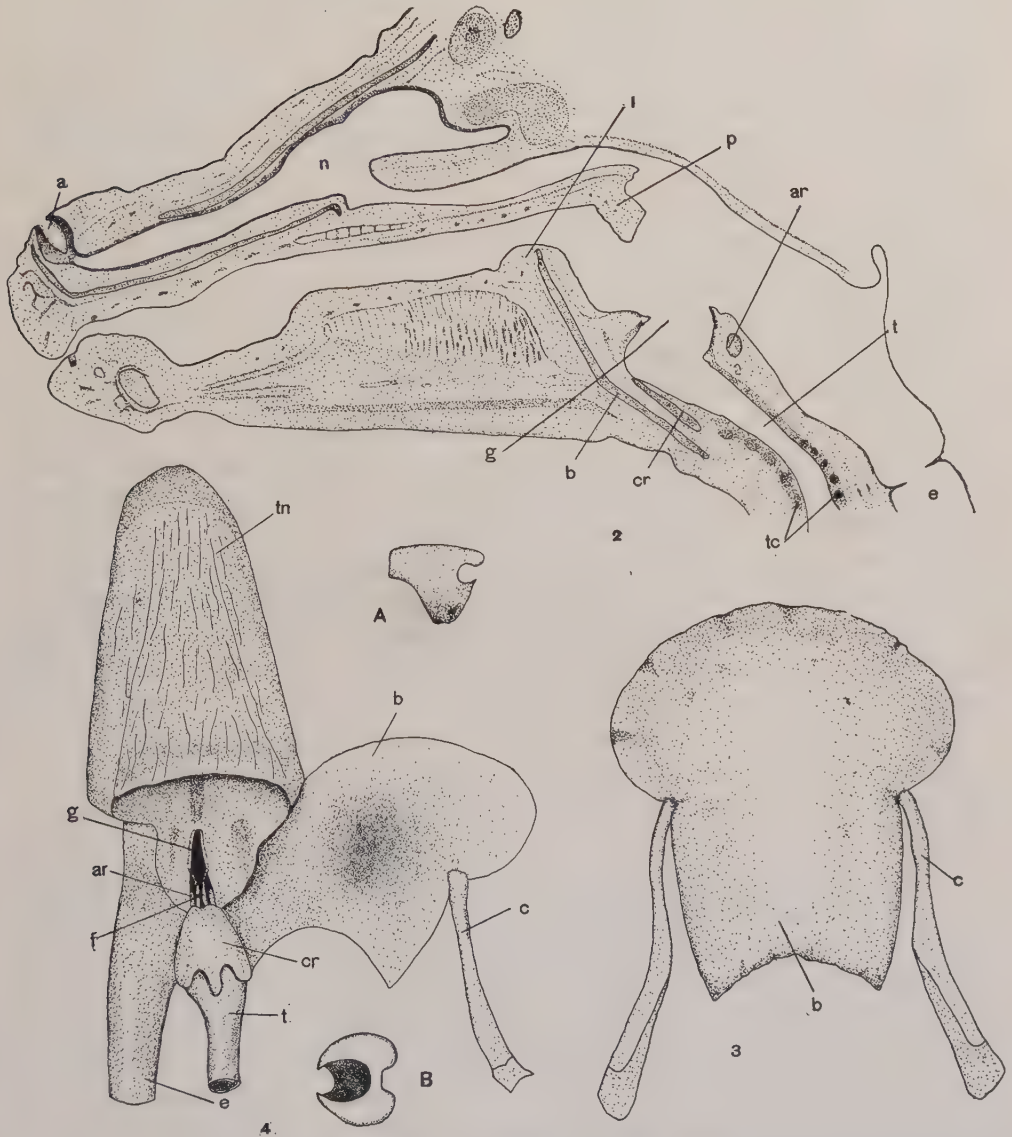


Fig. 2 Sagittal section of the head of an alligator embryo shortly before hatching, showing the palatal and lingual folds and other adjacent structures. Drawn with camera lucida.

Fig. 3 Ventral view of the hyoid apparatus of *A. mississippiensis*.

Fig. 4 Ventral view of the laryngeal apparatus of the alligator with hyoid drawn to right and ventral side uppermost. The larynx has been drawn caudad, exposing the glottis. The esophagus has been drawn to left of the trachae. Anterior ends of A and B are to the left.

THE LARYNX

Little work has been done on the larynx of the cold-blooded vertebrates, though most of the comparative anatomies give brief descriptions of this region. Nothing was found on the larynx of the Crocodilia. Owing partly to lack of material only the skeletal parts have been studied.

The larynx is shown in a ventral dissection in figure 4, together with the lower side of the tongue (tn) and the dorsal side of the basilingual plate (b), with its cornu (c), drawn to the right. The esophagus (e) is drawn to the left from its normal position dorsal to the trachea (t).

There is no epiglottis in the alligator and the vocal cords are commonly said to be absent; but they are, perhaps, represented by a long, deep-lying fold of tissue (f), on each edge of the glottis. Perhaps these folds might be called false vocal cords, as in some higher animals, since they are attached or closely adherent to the arytenoids.

Unless some sort of vibrating structure be present it is hard to understand how the young animals can make their characteristic, squeaky call or the old males their bellowing roar which may be heard, according to E. A. McIlhenny ('35), a distance of "at least 3 miles." He says: "This contraction forces the air with which his body is distended through the throat and out of the mouth which is open only from an inch to an inch and a half, and rapidly vibrates the membranous folds inside the glottis causing a tremendous volume of sound." The alligator has no syrinx.

There are three laryngeal cartilages; the two tiny, more or less shapeless arytenoids (ar) lying on each side of the posterior border of the glottis, and the large, ring-shaped cricoid (cr) sometimes called the thyro-cricoid.

Most of the comparative anatomies have little or nothing to say about the reptilian larynx. Adams ('38) says simply: "The reptilian larynx is formed by two arytenoid cartilages and a cricoid."

Hyman ('42) says: "... in the lateral walls of the larynx are the two arytenoid cartilages, small cartilages supporting the two triangular flaps which border the glottis. Posterior to the glottis is a ring-shaped cartilage, the cricoid, much wider ventrally than dorsally." Her figure of the larynx of *Sphenodon* shows the arytenoids to be much larger than would be expected from the description.

Kingsley ('25) names the same three cartilages in the reptiles but says: "The cricoid is usually an incomplete ring to which the arytenoids are attached. . . ."

In the alligator the arytenoids are, as noted above, tiny, more or less shapeless cartilages, attached to the anterior edge of the cricoid,

and lying on each side of the posterior region of the glottis, adjacent to, if not attached to, the folds of tissue mentioned above (false vocal cords?).

The cricoid (cr, fig. 4), as seen in a ventral view, is a fairly large cartilage, bluntly pointed at its anterior end, adjacent to the arytenoids, and with two deep notches and three rounded points at its posterior end; the median point is the longest.

Seen from the side (A, fig. 4), the cricoid shows one of the posterior notches and median point; the anterior border at the left of the figure, is much reduced in a dorso-ventral direction and the two sides are widely separated. The posterior border as seen in a dorsal view (B), extends dorsad and mediad to meet the border of the opposite side in a narrow, dorsal bridge. This bridge is so narrow that it was at first thought to be absent as described by Kingsley.

SUMMARY

This article discusses the anatomy of the hyoid and laryngeal skeleton of *Alligator mississippiensis*. The small arytenoids and much larger cricoid are similar to the structures appearing in other reptiles. The cricoid is a complete and not a broken ring as sometimes described. Attention is directed to the nice adjustment of the palatal and lingual folds by means of which the alligator is able to hold a struggling animal under water while preventing water from entering the trachea.

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A QUANTITATIVE STUDY OF TISSUE FLUID — LYMPH CELLULAR RATIOS

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INTRODUCTION

A great deal of information is available on the lymphocyte content of blood and thoracic duct lymph. Less information may be had on the cellular content of peripheral lymph and tissue fluid and there is at hand no quantitative data concerning the relation of the tissue fluid cellular content to that of peripheral lymph. Such information should be of value in regard to the problems of the "circulation of the lymphocyte" and the "fate of the lymphocyte". Former studies on lymphatic absorption from the peritoneal cavity led to the following attack on the problem.

The lymphatic plexus of the diaphragm is anatomically similar to lymphatic plexuses in many regions of the body. It is different in that it is in immediate proximity to the peritoneal cavity which Allen ('36) on anatomical grounds and Maurer et al. ('40) from chemical studies have likened to a tissue space containing a variable amount of tissue fluid. This is an ideal location for a quantitative study of this kind. Here can be obtained, without trauma or contamination, serous or tissue fluid for cellular analysis and at the same time from the diaphragmatic plexus lymph which a few seconds before was serous fluid. It is thus possible in this location to determine the effect of passage through the lymphatic wall on the cellular content of the normally occurring serous fluid or upon any cellular suspension injected into the peritoneal cavity.

This work was initially undertaken with the idea of obtaining data pertinent to the problem of the circulation of the lymphocyte but the scope of the problem was extended to include data on the lymphatic absorption of other cell types, namely, erythrocytes, polymorphonuclear leucocytes and cellular suspensions of bone marrow and lymph nodes. The data will be considered first in relation to the circulation of the lymphocyte; second, from the standpoint of the permeability (or penetrability) of the lymphatic wall; and third, in regard to the question of intravascular versus extravascular origin of cells of the blood.

MATERIAL AND METHODS

Large rabbits of 5 to 7 pounds were used exclusively. The animals were killed with a blunt instrument, bled and the thorax and abdomen opened widely. One or more of the large collecting lymphatics which converge toward the sternum on either side of the superior surface of the diaphragm were nicked with a razor blade and the extruding drop of lymph drawn up in a standard blood pipette. In some instances it was necessary to stroke the lymphatic gently in order to express the lymph for sampling. The liver was then retracted and samples of

TABLE 1

Determinations of leucocytes per cubic millimeter in serous fluid and diaphragmatic lymph in the rabbit.

RABBIT NO.	PERITONEAL FLUID	LYMPHO-CYTES	DIAPHRAGM. LYMPH	LYMPHO-CYTES	ABSORPTIVE RATIOS
1	266		55		4.8:1
2	1,180	50%	300	61%	3.9:1
3	2,400		400		6.0:1
4	3,200	57%	400	63%	8.0:1
5	3,900	89%	500	80%	7.8:1
6	19,750	95%	2,000	95%	9.1:1
7	5,500	93%	900	95%	6.1:1
8	1,900	88%	525	85%	3.6:1
9	1,603		320		5.0:1
10	420		166		2.5:1
11	866		400		2.2:1
12	1,100		880		1.3:1
13	950		450		2.1:1
Average of ratios					4.8:1

serous fluid were secured immediately beneath the same half of the diaphragm from which the lymph was obtained. Smears of this lymph and serous fluid were made and stained with Wright's stain. Standard counts were also made in a blood counting chamber, 16 squares being counted instead of the usual 4 squares. Table 1 records the data on the cellular content of the normally occurring serous fluid and on lymph, the lymph being that which was absorbed immediately before the death of the animal.

Table 2 gives the data on the absorption of erythrocytes. In each animal 10 cc. of blood was drawn from the heart, heparinized¹ and injected intraperitoneally. Each animal was killed about 20 minutes after injection. This time interval was allowed for thorough mixing in the

¹ Furnished by Dr. Y. SubbaRow, Lederle Laboratories.

cavity, not for absorption, since absorption and passage through the diaphragm is a matter of seconds rather than minutes. Lymph from both halves of the diaphragm and serous fluid from beneath each half were sampled.

TABLE 2

Simultaneous determinations of red blood cells per cubic millimeter in peritoneal cavity and diaphragmatic lymphatics, absorption effected by normal diaphragmatic movements.

RABBIT NO.	PERITONEAL FLUID	DIAPHRAGMATIC LYMPH	ABSORPTIVE RATIOS
14	1,390,000	760,000	1.8 : 1
	1,350,000	730,000	1.8 : 1
15	1,150,000	450,000	2.5 : 1
	1,060,000	440,000	2.4 : 1
16	2,590,000	1,900,000	1.4 : 1
	2,640,000	1,790,000	1.4 : 1
17	4,160,000	2,560,000	1.6 : 1
	4,190,000	2,910,000	1.4 : 1
18	3,230,000	2,620,000	1.2 : 1
	3,320,000	2,530,000	1.3 : 1
Average of absorptive ratios			1.68 : 1

TABLE 3

Simultaneous determinations of red blood cells per cubic millimeter in peritoneal cavity and diaphragmatic lymphatics, absorption being effected by massage.

RABBIT NO.	PERITONEAL FLUID	DIAPHRAGM. LYMPH	ABSORPTIVE RATIOS
19	2,650,000	2,590,000	1.01 : 1
20	4,300,000	4,420,000	1.03 : 1
21	5,270,000	5,230,000	0.99 : 1
22	9,962,000	9,197,000	1.08 : 1
Average of ratios			1.04 : 1

Table 3 shows the data on erythrocyte absorption effected by massage. Immediately after the death of the animal the abdomen was opened widely, 10 cc. of the animal's own heparinized blood was poured between diaphragm and liver. The abdomen was closed with hemostats, massaged for 1 or 2 minutes, following which lymph and serous fluid were sampled. In one experiment (rabbit 22) erythrocytes were concentrated in the centrifuge.

Table 4 gives the data on the absorption of polymorphonuclear leucocytes. A fecal peritonitis was produced by the method of Steinberg

and Snyder ('29), in order to produce an appreciable number of polymorphonuclear leucocytes. The animal was killed at the end of 4 hours. Samples of lymph were taken from both halves of the diaphragm and serous fluid from beneath each half.

TABLE 4

Simultaneous determinations for polymorphonuclear leucocytes per cubic millimeter in serous fluid and in diaphragmatic lymph in fecal peritonitis.

RABBIT NO.	PERITONEAL FLUID	DIAPHRAGM. LYMPH	ABSORPTIVE RATIOS
23	42,850	18,500	2.3: 1
24	46,600	7,750	6.1: 1
24	64,000	7,000	8.3: 1
	20,000	9,150	2.2: 1
25	11,300	6,500	1.7: 1

Differential — 90% to 95% polymorphonuclear leucocytes for all counts.

TABLE 5

Simultaneous determinations of marrow cells per cubic millimeter in suspension in peritoneal cavity and diaphragmatic lymphatics.

RABBIT NO.	CELLS PER CMM. IN PERITONEAL CAVITY	CELLS PER CMM. IN DIAPHR. LYMPHATICS	SOURCE OF CELLULAR SUSPENSION	ABSORPTIVE RATIOS
26	250,000 ¹	140,000 ¹	Marrow	1.8: 1
	40,000	13,000	Marrow	3.1: 1
27	34,800	13,700	Marrow	2.5: 1
28	21,050	10,350	Lymph nodes	2.0: 1
29	40,300	28,300	Lymph nodes	1.5: 1
30	33,000	9,425	Lymph nodes	3.5: 1
31	38,000	13,000	Lymph nodes	2.9: 1

¹ Red blood cells; all others WBC.

Suspensions of bone marrow were prepared by macerating tibial and femoral marrow in about 7 cc. of normal saline and suspension of lymphocytes were prepared, by the same technique, from mesenteric nodes. These suspensions were injected intraperitoneally and the animals were killed after 20 minutes and the fluids sampled. This data is recorded in table 5.

DISCUSSION

The diaphragmatic lymph which was sampled had passed through no lymph node and may accordingly be considered peripheral lymph. The average cell count is 586 WBC per cubic millimeter with a range of 0 to

2500 WBC per cubic millimeter. Baker ('32) has reported an average of 699 WBC per cubic millimeter with a range of 55 to 2000 WBC per cubic millimeter for peripheral intestinal lymph. Yoffee and Drinker ('39) report a mean of 550 WBC per cubic millimeter for peripheral leg lymph. The leucocyte content of diaphragmatic lymph is accordingly quite similar to that of peripheral lymph in other locations.

It is apparent from table 1 that the tissue fluid—lymph cellular ratios are variable and there are on the whole, approximately five times as many leucocytes in tissue fluid as in lymph. It would probably be best not to stress this figure as an average until more is known about the factors which are responsible for variations in the absorptive ratios. The constant discrimination against the absorption of lymphocytes as well as its variability in different experiments is of considerable interest in regard to the problem of the "circulation of the lymphocyte" and may possibly have a bearing on the question of the "fate of the lymphocyte".

Table 1 shows extremes in absorptive ratios of from 9.1 to 1 and 1.3 to 1. In seeking for an explanation for this variability it was noted that the last four animals in this series had considerably more free fluid in the peritoneal cavity than the others. These four (rabbits 10, 11, 12, 13) showed the highest absorptive ratios (near 1 to 1). Erythrocytes (table 2), marrow and lymph node cells (table 5), all of which were suspended in abundant fluid also show high absorptive ratios. Similarly it was noted in the experiments on the absorption of polymorphonuclear leucocytes (table 4, rabbits 23, 24), that there was an unequal distribution of fluid beneath the two halves of the diaphragm and that the higher absorptive ratios pertain to the side showing excessive fluid. As a further test of the effect of fluid suspension, 10 cc. of saline was injected intraperitoneally into rabbit 25 (table 4) 30 minutes before the death of the animal. This animal shows the highest absorptive ratio (1.7 to 1) in this series.

A second probable factor in the absorptive ratios is the pressure differential² which produces absorption. The experiments in which absorption was effected by massage (table 3) show approximate 1 to 1 ratios. In these experiments the diaphragmatic excursions, particularly the upward ones, were greater than in the living animals. This would produce greater negative intralymphatic pressures and more marked

² There has been as yet no exact measurement of the minimum pressure differential necessary to force a suspended particle through an intracellular opening. Allen ('38) has demonstrated relative negative pressures in lymphatics of several tissues and measured a maximum of minus 3.0 cmm. of water in lymphatics of the frog's web.

stretching of the lymphatic wall. Final evaluation of these factors must await upon specially designed experiments.

The discussion of the second factor is based on the assumption that the leucocyte is absorbed as a non-motile body. The data presented here indicate that this is the case. There is no marked difference in the differential counts on serous fluid and lymph and in comparable experiments the absorptive ratios of leucocytes are as high as the absorptive ratios of erythrocytes.

Allen ('36), injected frog erythrocytes intraperitoneally in mice and demonstrated histologically that during absorption erythrocytes pass through peritoneal stomata and deep to these, between lymphatic endothelial cells. The mechanics of lymphatic absorption were described by Allen and Vogt ('37). It is concluded that the leucocyte is absorbed in a similar manner. This is contrary to the general assumption that leucocytes are absorbed by migration through the endothelial wall of lymphatics and to the conclusion of MacCallum ('03), who studied the diaphragmatic lymphatics histologically following the intraperitoneal injection of particulate matter and interpreted the presence of particle laden phagocytes as due to active migration.

There is, accordingly, nothing specific in the absorption of the lymphocyte as a cell. Erythrocytes, polys, monocytes, lymphoid and myeloid cells may be absorbed with equal readiness. In one sense, the cellular content of peripheral lymph may be thought of as an accident. The lymphatic capillary has no selective affinity or peculiar attraction for the lymphocyte during active absorption nor does it when absorption is not in progress (Clark and Clark, '37). The lymphocyte is the most common cell in peripheral lymph because it is the most common cell in tissue fluid.

Bunting and Huston ('21) and Blalock et al. ('37) have shown that ligation of the thoracic duct results in a rapid diminution in blood lymphocytes, but opinions vary as to sites of removal and ultimate fate of these cells (Yoffee and Drinker, '39; Ehrich, '45). Direct observation by Clark and Clark ('32) shows that many lymphocytes migrate into connective tissues from blood capillaries. While it is conceivable that lymphocytes under certain conditions, as pertain for erythrocytes in table 3, might be absorbed in a 1 to 1 ratio, the data in table 1 indicates that lymphocytes are always discriminated against in the absorptive process, that the lymphatic endothelium acts as a partial barrier to the "circulation of lymphocytes" from blood to lymph. Since many lymphocytes are constantly denied reentry into the lymphatics and since reentry is non-specific and in a sense an acci-

dent, it becomes more likely that the lymphocyte serves its main function, degenerates or changes into other cell types somewhere between blood and lymph capillaries.

Allen and Vogt ('37), from a study of the mechanism of lymphatic absorption and particularly in consideration of the almost instantaneous passage of suspensions through the lymphatic wall, have felt that tissue fluid and lymph must be quite similar. This view has been stressed repeatedly by Drinker and Field ('33) from chemical studies of lymph and tissue fluid. This would seem to be the obvious conclusion to draw from a consideration of table 3 which shows a 1 to 1 ratio of erythrocyte absorption. The other data, however, indicate that the subject is too complicated to be summarized in so simple a conclusion since lymph and tissue fluid are not normally identical in cellular composition. All our data support the contention that the lymphatic wall is a penetrable rather than a semipermeable membrane as maintained by Hudack and McMaster ('33).

Current theories of origin of the cells of the blood have been concerned with the necessity of getting the newly formed cells into the blood stream. If cells arise extravascularly they must pass in some way through an endothelial wall. If they cannot conceivably pass through the wall they must arise intravascularly from specialized endothelium.

Maximow ('10) believed that granulocytes enter the sinusoids by active migration while erythrocytes enter through tears in the sinus wall. Doan, Cunningham and Sabin ('25) believe that the vascular system of bone marrow is entirely closed and that erythrocytes arise within collapsed intersinusoidal capillaries from endothelium. Drinker ('21) states that under certain conditions the vessels communicate freely with blood islands of the reticulum and Drinker, Drinker and Lund ('22) hypothesize a growth pressure as a means of delivery of erythrocytes through an eroding of the endothelial wall and attribute to the closely packed cells of a developing focus a quality of adhesiveness that makes a cellular limitation to the injection mass other than endothelium. Isacacs ('30) noted that in fresh marrow the more mature erythrocytes were in a liquid intercellular substance and enter the circulation by temporary solution of the capillary wall. All agree that it is quite difficult to observe exactly what happens to the thinned out endothelium near erythrogenic foci. There does not seem to be any disagreement concerning the ability of granulocytes to migrate into the marrow sinuses by their ameboid activity. There is an open question here in consideration of leukemia. Can myeloblast and myelocytes

actively migrate through the endothelium of sinuses and if so why do they not appear normally in the blood stream? No one has given a satisfactory answer. Almost all observers agree however that normally the cells in contact with the sinus wall are the more mature cells in the series and that only under stress do the younger forms appear.

From a study of smears it was noted that every cell of the hematopoietic series, with the exception of the giant cell, passed through the endothelial wall, roughly in proportion to its concentration in the suspension. On the basis of these experiments it is possible to offer a uniform theory for the delivery of marrow cells to the circulation. If it may be assumed that the endothelium of the sinuses of bone marrow is anatomically and physiologically similar to lymphatic endothelium, then any cell of the myelopoietic or erythropoietic series *in suspension* may be forced through intercellular openings by a pressure differential and thus appear in the open circulation.

As in the case of bone marrow cells, suspension prepared from lymph nodes (table 5) pass readily through the intercellular openings of lymphatic endothelium. Hence there can no longer be any theoretical contention that an open vascular system is necessary in certain locations, either in the sense of temporary breaks, tears, or solutions of endothelium, or in the sense of endothelium becoming continuous with reticulum.

SUMMARY

1. Cell counts were made simultaneously on serous fluid and diaphragmatic lymph and the same procedure was repeated, using whole blood, suspensions of lymph nodes and marrow and the fluid of inflammation.

2. Every type of cell which occurs normally in tissue fluid and blood will penetrate lymphatic endothelium. We believe we have identified every cell of the hematopoietic series except giant cells, in lymph absorbed from marrow suspensions injected intraperitoneally. Likewise, the entire series of lymphoid cells will pass through endothelium.

3. Tissue fluid — lymph cellular ratios show that there is discrimination against the absorption of cells. This discrimination is not at the expense of any one type of cell and varies in different animals.

4. The data indicate that free suspension of cells in fluid and strong tissue movements favors the absorption of cells.

5. Tissue fluid and lymph are not identical in cellular content.

6. As an average, for each 167 RBC per cubic millimeter red blood cells in suspension in the peritoneal cavity, 100 per cubic millimeter are found in diaphragmatic lymph. The data on the absorption of leuco-

cytes is too variable to be dealt with as an average, but in many instances leucocytes were absorbed as readily as erythrocytes.

7. During active lymphatic absorption, leucocytes are absorbed as non-motile bodies.

8. The demonstration of the penetrability as a normal attribute of endothelium in one location makes it possible to reconcile the anatomical concept of closed endothelium with the physiological necessity for an open circulation in certain locations. The implications of these findings in regard to bone marrow and lymph nodes are discussed.

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HORMONAL INDUCTION OF MATING RESPONSES IN A RAT WITH CONGENITAL ABSENCE OF GONADAL TISSUE¹

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INTRODUCTION

The possible role of prenatally secreted gonadal hormones in the differentiation of the vertebrate reproductive system has been variously estimated by different workers. According to one school of thought (Willier, '39; Greene, '44), endocrine products of the embryonic ovary or testis exert a controlling influence over the developmental organization of the accessory sexual structures. In opposition to this point of view stands the theory that differentiation and development of the reproductive system occurs independently of gonadal hormones and is inherent in the genetic constitution of the genetically-determined sexes (Moore, '44a, '44b).

Studies of courtship and mating behavior in lower mammalian species, particularly the rat, have shown that such activities are displayed in complete form and at normal frequency by males and females which have been raised in isolation from weaning to adulthood (Stone, '22; Beach, '42a). At this phylogenetic level practice and individual experience in the specific sexual responses apparently are nonessential to the biologically effective execution of copulatory behavior. For those species in which this situation obtains, it has been suggested that the arousal, maintenance, and manifestation of sexual excitement is mediated by nervous mechanisms whose organization is genetically determined (Beach, '42b). It has furthermore become evident, in some species at least, that the nervous organization for mating behavior is complete at birth or shortly thereafter, although the hormonal facilitation essential to the activation of these neural circuits does not normally become available until late adolescence (Beach, '42b).

If such is indeed the case, the nervous mechanisms responsible for overt mating reactions are to be regarded as an integral unit in the reproductive system, and must be considered accessory sexual elements comparable to such other structures as the uterus, vagina, penis, vas

¹ This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

deferens, etc. It is therefore to be expected that the differentiation of the behavioral mechanisms will depend upon the same controlling forces, whatever their nature, that direct the developmental organization of the more commonly recognized units in the reproductive complex.

OBSERVATIONS

While spaying a number of virgin female rats ² 90 to 100 days of age we discovered one individual in whom no ovarian tissue could be detected. Further examination disclosed the absence of uterine horns, and the external vagina was found unruptured, appearing comparable to that of a 25-day female.

The rat was set aside until the incisions were completely healed and 3 weeks after operation hormones were administered to induce estrus. The course of endocrine treatment adopted was that previously proven capable of eliciting normal sexual behavior in spayed females from our stock (Beach, '42c). An intramuscular injection of 500 R.U. of estradiol benzoate was followed after a 48-hour interval by the injection of 0.5 mg. of progesterone ³; and tests for sexual activity were conducted 12 hours later.

Behavior. When placed with a sexually vigorous male the injected animal displayed all elements of the mating pattern shown by a female in normal estrus. She exhibited marked precopulatory interest in the male, investigating him repeatedly and responding to his reciprocal explorations with the short darting run terminating in a semi-crouch which characterizes the behavior of the receptive female. In a space of 5 minutes the male mounted and clasped the female fourteen times. In every instance the female responded promptly with the assumption of a full lordosis position, head elevated, pelvic region raised to present the vaginal opening and facilitate intromission. In addition the female once showed lordosis when the male placed his forepaws on her back without mounting. This extreme readiness to exhibit lordosis and opisthotonus typifies the highly receptive female, and is normally seen for only a few hours in each estrous cycle at a time when heat is maximal (Ball, '37; Young, Boling and Blandau, '41). During

² The rat colony at the American Museum of Natural History was originally derived from Wistar stock, but in a period of approximately 15 years tame females have occasionally been bred to trapped wild males with the presumed increase in heterozygosity. In addition there has been a consistent tendency to breed completely pigmented animals so that albinos occur infrequently.

³ The hormone preparations, which were provided through the courtesy of Dr. Max Gilbert of Schering Corporation, Bloomfield, New Jersey, are commercially known as Progynon-B, and Proluton.

copulation and at times when the male was conducting his investigations the female displayed the very rapid head shaking which produces a vibratory movement of the ears. Ear vibration is the third element of the normal estrous pattern.

Reproductive anatomy. Upon sacrifice and dissection the tentative conclusions based upon biopsy data were confirmed. Although the entire abdominal cavity was carefully explored no trace of gonadal tissue was revealed. The masses of fatty tissue normally associated with the ovarian capsule and the uterine horns were absent; and the horns themselves were totally lacking. The external vagina was still closed, despite the hormone treatment, but it ruptured easily when a probe was inserted. Internally the vagina entered a short, abortive, uterine-like structure which was 8 mm. in length and ended blindly at about the point where bifurcation would normally occur. Histological section showed that the abbreviated organ was truly a uterus; and in its distal portions two separate canals were evident. The endometrial glands were few and appeared to be inactive, but poor fixation rendered accurate interpretation difficult. On one side a tube led from the region of the urogenital sinus to the tip of the blind uterine pouch. Sections of this duct were difficult to study (fixation poor), but gave no indication of either Wolfian or Mullerian origin.

INTERPRETATION

We are inclined to believe that the rat here described was a genetic female in which gonadal tissue failed to differentiate. The same chromosomal failure, or one associated with it, may be presumed responsible for absence of fully developed uteri. It should be pointed out, however, that the vagina and a portion of the uterus did differentiate and grow to the infantile, postnatal level.

Our records of the animal's behavior under the influence of injected hormones convincingly show that the nervous mechanisms for feminine mating responses were completely organized and capable of mediating normal sexual behavior as soon as the appropriate sensitizing chemical agents were introduced.

These data may be compared with Riddle's reports of courtship and treading in pigeons with congenital lack of gonadal tissue ('24-25).

It would thus appear that differentiation and development of the neural structures controlling sexual behavior occurred in this case despite the absence of any gonadal hormones. This suggests that such secretions do not act as embryonic organizers insofar as the behavioral mechanisms are concerned. On the contrary, it may be proposed that

the ontogenetic development of these mechanisms is, as Moore ('44a) has suggested for the remainder of the reproductive system, inherent in the chromosomal constitution of the genetically determined sex of the organism. Insofar as overt behavior is concerned, the gonadal hormones may not come into play until, just before maturity, they reach a concentration sufficient to alter the sensitivity of the pre-organized neural mechanisms, and markedly increase the responsiveness of the latter to exteroceptive stimulation.

SUMMARY

One female rat with congenital absence of gonadal tissue and uterine horns was observed to display normal feminine mating behavior when placed with a male following the injection of estrogen and progesterone. The bearing of these findings upon theories of embryonic differentiation of the central nervous mechanisms for copulatory activity is discussed.

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FUNCTIONAL SIGNIFICANCE OF THE INSERTIONS OF THE EXTENSOR COMMUNIS DIGITORUM IN MAN¹

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SIX FIGURES

Operative correction of finger deformities requires a detailed knowledge of the anatomy and an understanding of the relation between structure and function. This relation is complicated in the action of the fingers because of the great number of factors involved. The flexion of the three phalanges is well understood and the relative role of the long flexors and the intrinsic muscles of the hand has been satisfactorily explained. However, the extension of the phalanges is more involved. There is still no agreement as to the exact distribution of extensor action produced by the extensor communis digitorum and the intrinsic muscles. There is also no complete agreement as to the insertion of the tendon of the common extensor into the base of the proximal phalanx of the finger. The nature of this insertion would be of little importance were it not for the fact that many investigators, following the classic description of Duchenne (1867), have regarded it as essential to explain the action of the extensor muscles. Duchenne believed that the extension of the proximal phalanx with a corresponding flexion of the two distal phalanges is due to two factors: integrity of the long flexors and the existence of a special tendonlike band which fixes the volar aspect of the extensor communis tendon to the base of the proximal phalanx.

A review of the literature discloses a wide disagreement concerning this insertion which Duchenne believed was fundamental to the mechanism of finger action. Poirier and Rouvière ('12), Poirier and Baumgarten ('20), Testut and Jacob ('22), Hauck ('23), Deaver ('26), Rouvière ('27), Mason ('30), Montant and Baumann ('37, '38), and Bunnell ('42, '44) are essentially in agreement with Duchenne. On the

¹ The author wishes to acknowledge his indebtedness to the late H. C. Raven of the American Museum of Natural History for the chimpanzee and baboon material used in this study.

other hand, Cloquet (1834), Gruveilhier (1843), Sappey (1888), Testut ('28), Grant ('40) and Lockhart ('43) do not mention this insertion.

In a previous article the writer described the insertion of the extensor communis into the base of the proximal phalanx as being constant (Kaplan, '39). Since then, however, it was frequently observed that a number of fingers did not show this insertion. This observation led to the present study, the purpose of which was to establish the exact nature and incidence of this insertion and its relation to the dynamics of finger action.

MATERIAL

The hands of twenty-three fresh and embalmed human cadavers were dissected. Twenty-two of these were white and negro adults of both sexes. The remaining cadaver was a premature 8 months white baby. In addition, the hands of a gorilla, chimpanzee and baboon were also studied for comparison. A series of anatomical experiments was performed to elucidate the function of the exposed parts.

Since manipulation of dissected material is an artificial procedure at best, the muscles of the hands of more than 100 living subjects were stimulated electrically. The results obtained in this manner were used to supplement those observed by dissection.

METHODS AND OBSERVATIONS

1. *Dissection*

The extensor and dorsal aponeurosis were dissected and examined in situ in all the fingers except the thumbs. The dorsal aponeurosis was completely removed from some of the fingers and its volar surface was examined. Other fingers were dissected for better visualization of the structures over the metacarpophalangeal joint. In these the extensor tendon was severed transversely over the middle of the proximal phalanx and the middle third of the corresponding metacarpal, and was completely separated from the lateral connections with the intrinsic muscles. The dorsal aponeurosis could then be lifted from the dorsum of the proximal phalanx and the dorsum of the metacarpal. The relation of the volar surface of the extensor tendon to the metacarpophalangeal joint was then studied. Here careful observation was necessary because it is easy to sever the insertion running to the base of the proximal phalanx without being aware of it, and it is equally easy to create a non-existent insertion.

As a result of these dissections it was found that the extensor communis tendon forms a roof over the capsule of the metacarpophalangeal

joint. The tendon is loosely connected with the capsule. Distal to the capsule the tendon divides into three bands which diverge over the proximal phalanx. The middle band continues the course of the tendon, forms an inseparable part of the capsule of the proximal interphalangeal joint and inserts into the base of the middle phalanx where it ends. The two lateral bands pass to the sides of the proximal phalanx, cross the proximal interphalangeal joint slightly dorsal to the transverse axis of this joint and then pass to the dorsum of the middle phalanx to unite over its distal third. From this point on the two lateral bands form a wide common ribbonlike tendon which serves as a dorsal wall for the distal interphalangeal joint and terminates on the base of the distal phalanx. The lateral bands are joined by the tendons of the intrinsic muscles.

Transverse fibers originating from the volar surface of the extensor tendon can be seen following a course toward the sides of the metacarpophalangeal joint and its collateral ligaments. Sometimes these bands seem to be running toward the deep intermetacarpal transverse ligament, as described by Bryce ('23) and other anatomists. This arrangement was found in all the fingers examined.

In a number of fingers an additional insertion was seen distal to the transverse fibers described above. This consisted of a short fibrous slip made up of several separate strands or forming a single band which ran obliquely from the palmar surface of the middle band of the extensor tendon distally toward the dorsum of the base of the proximal phalanx (fig. 1). This band corresponds to the insertion of the extensor communis tendon into the base of the proximal phalanx described by Duchenne (1867). It was never found to be tendinous, as represented by Deaver ('26). In the same hand it could be found in one, two or even three fingers and be absent in the others. It was found in only 54 or 38.5% of 140 fingers examined. There was no apparent relation between the presence of this insertion and the age, sex, color and the general muscular development of the subject.

The relation of the interossei and lumbrical muscles to the lateral bands of the common extensor tendon was studied in situ. In the space between the metacarpal heads the dorsal and palmar interossei form a flat conjoined tendon directed anteroposteriorly, which continues its course over the dorsolateral aspect of the capsule of the metacarpophalangeal joint. At the base of the proximal phalanx the tendon divides into two parts: an anterior and posterior. The anterior forms a strong insertion to the lateral tubercle of the base of the proximal phalanx. The posterior continues distally, parallel to the lateral band of the

extensor tendon and superficial to it. Just proximal to the metacarpophalangeal joint the interosseus tendon sends a flat expansion toward and over the middle band of the extensor tendon on the dorsum of the proximal phalanx. This, together with a similar structure formed by the interosseus on the other side of the finger, covers the extensor



Fig. 1 Dissected human hand. The extensor tendon is luxated to the radial side of the index finger, showing the insertion of the extensor communis to the base of the proximal phalanx, as observed in 35.8%.

tendon (fig. 2). The rest of the interosseus tendon continues its course parallel to the lateral band of the extensor toward the proximal interphalangeal joint. At this point it sends a few spiral fibers dorsal to the lateral band of the extensor tendon. The spiral bands cross the lateral

bands of the extensor obliquely to insert in the median band at the base of the proximal phalanx (fig. 2). The interosseus tendon continues its course further, running parallel to the lateral band and terminates together with the lateral band, into the base of the distal phalanx. The tendon of the lumbrical muscle joins the interosseus on the radial side and runs parallel to the interosseus and radial to it and the lateral band of the extensor tendon.

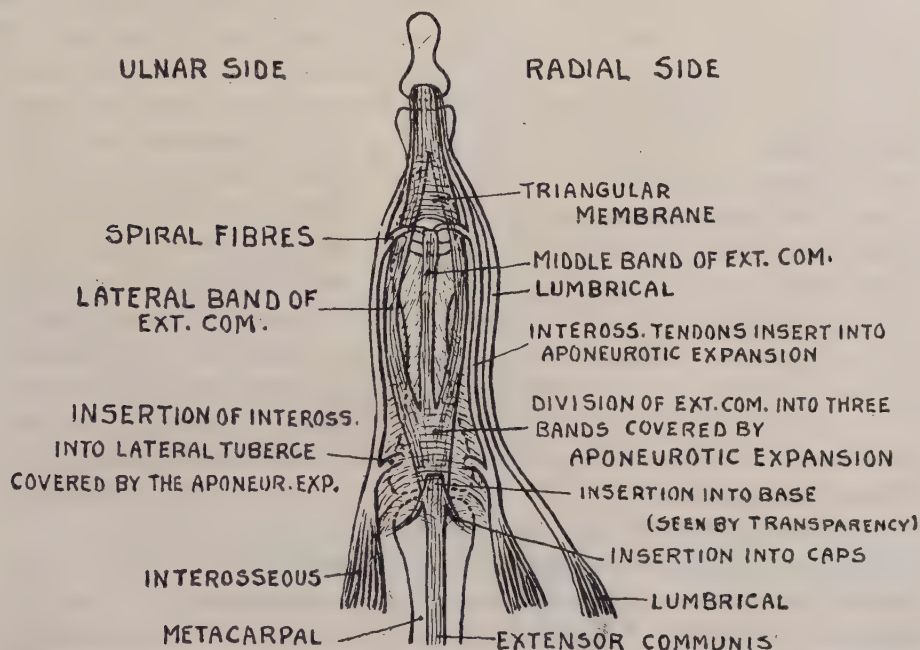


Fig. 2 Semi-diagrammatic illustration of the extensor apparatus of a finger with the tendons of the interossei and the lumbricals artificially separated.

The insertion of the extensor tendon and of the tendons of the interossei and lumbrical muscles into the phalanges of the primate hand were observed to be essentially the same as in man.

2. Experiments on the cadaver

Muscle action was simulated in the dissected material by producing traction on the exposed tendons. In addition to the study of the actions of the intact tendons, the effect of section of the extensor apparatus across the proximal, middle, and distal phalanges and over the corresponding joints was also investigated. The experiments and the pertinent facts observed are summarized below.

1. The hand was placed in complete flexion at the wrist and fingers. Traction was then applied to the common extensor tendon proximal to the metacarpophalangeal joint in a cephalad direction. This produced extension of the distal, then middle and proximal phalanges for a short distance only. When the proximal phalanx approached the plane of the metacarpal, the distal two phalanges began to flex.

2. The flexor sublimis and profundus tendons were severed and traction was applied to the common extensor as above. Irrespective of the position of the hand, the proximal, middle and distal phalanges all extended. However, hyperextension was obtained only at the metacarpophalangeal joint and could not be obtained in the two distal phalanges.

These experiments showed that extension of the two distal phalanges, after the proximal phalanx is extended, was prevented by the two long flexors of the hand. It also indicated that, even with the two long flexors severed, extension of the two distal phalanges could be obtained but hyperextension of the two distal phalanges could not be accomplished.

3. The long flexors of the finger were severed. The connecting band between the common extensor and the base of the proximal phalanx, as well as the fibers connecting this tendon with the capsule of the metacarpophalangeal joint were divided. Traction was then applied to the common extensor tendon in a proximal direction. This produced an extension of the three phalanges. Further traction produced hyperextension of all the phalanges.

This group of experiments showed that the separation of the common extensor from the metacarpophalangeal area eliminated the pull that the common extensor normally exerts on the proximal phalanx directly or through the capsule. Traction applied to the extensor tendon transmitted the force equally through the middle band to the base of the middle phalanx and through the lateral bands to the base of the distal phalanx, thus producing extension of all phalanges.

4. The extensor communis tendon was severed across the proximal phalanx, proximal to the insertion of the intrinsic muscles. Traction was applied to the distal cut end of the extensor. This produced hyperextension of the proximal phalanx, the two distal phalanges remaining flexed in all positions of the hand. If traction was then applied to the intrinsic tendons, the two distal phalanges extended.

5. The extensor tendon was pulled to bring the proximal phalanx in line with the corresponding metacarpal. The pull was then released and traction was applied to the intrinsic muscles. This traction produced a strong flexion of the proximal phalanx with a corresponding extension of the two distal phalanges.

These experiments showed that the intrinsic muscles are extensors of the two distal phalanges not only when the proximal phalanx is extended but also when the proximal phalanx is flexed.

6. The length of the full excursion of the tendons of the flexor profundus and extensor communis from complete flexion to complete extension was measured. The relation of full excursion length of the flexor profundus to the excursion length of the extensor communis at the metacarpophalangeal joint was found to be approximately 4:3. At the proximal interphalangeal joint the relation of the excursion length of the flexor profundus tendon to the excursion length of the lateral bands of the extensor communis was approximately 4:1.

This relation is important because it shows that full extension of the three phalanges cannot be obtained at the metacarpophalangeal joint by the extensor communis tendon as compared with the flexor profundus tendon which can produce full flexion of all the phalanges. Full extension is produced by the additional traction exerted by the action of the intrinsic muscles through the lateral bands in the approximate proportion of 1:4 to the flexor profundus.

3. *Experiments with electric stimulation*

A faradic stimulator was used. One of two electrodes was applied between the metacarpal spaces with the hand in the physiological position of rest. Upon stimulation in this position, the corresponding finger showed strong flexion of the proximal phalanx with simultaneous extension of the two distal phalanges. If, however, simultaneous mild stimulation was applied to the common extensors as well as the interossei muscles, a strong extension of all the three phalanges took place.

If the common extensors are stimulated while the hand forms a fist, extension always started in the distal and middle phalanges and rapidly extended to the proximal phalanges. However, as soon as the proximal phalanges started to extend, the corresponding two distal phalanges began to flex.

When the stimulation was applied in the second or third metacarpal space with the other electrode over the ulnar nerve at the wrist, the middle finger in addition to strong flexion of the proximal phalanx and extension of the two distal phalanges, produced radial or ulnar deviation directed to the stimulated intermetacarpal space. Stimulation of the first intermetacarpal space produced only flexion of the proximal phalanx of the index finger with extension of the two distal phalanges without radial deviation of this finger. Occasionally, one could see a slight radial deviation of the index finger.

Although the limitations of electrical stimulation are well recognized, this method of investigation confirmed the facts observed by anatomical experimentation.

DISCUSSION

Although the insertion of the extensor communis digitorum tendon into the proximal phalanx has been described by both anatomists and clinicians, its functional significance has not been clear nor has its incidence been recorded. In the present study the incidence has been established as 38.5%. It has also been found that this insertion is not functionally significant. Its absence does not affect the extension of the phalanges of the fingers. The role of the insertion appears to be the reinforcement of the connection between the extensor tendon and the capsule of the metacarpophalangeal joint. This connection between the tendon and the capsule is formed essentially by short fibrous fas-

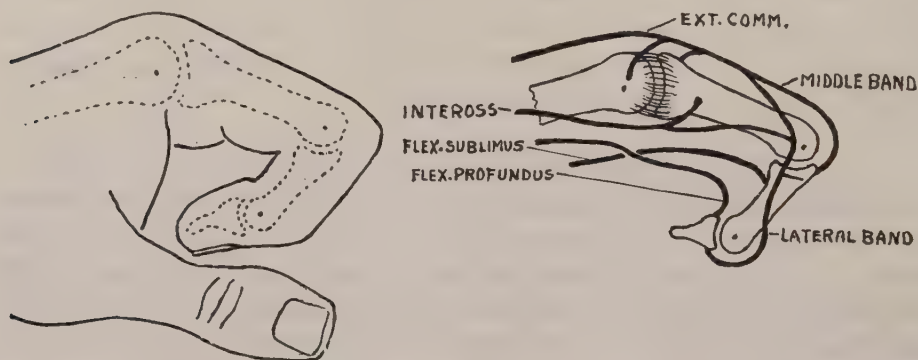


Fig. 3 Diagrammatic illustration of the index finger in flexion with the tendons represented by heavy black lines. In this position the common extensor acts through the middle band, extending the two distal phalanges until their extension is restricted by the action of the flexors and the tension of the insertion into the proximal phalanx or into the capsule of the metacarpophalangeal joint. Both insertions are represented. The center of rotation of the joints is represented.

ciculi which permit a limited proximal and distal movement of the tendon. This differs from the condition seen at the proximal and distal interphalangeal joints. Here the capsule of the interphalangeal joint is intimately connected with the extensor tendon and does not allow independent movement of the tendon.

In extension of the finger the extensor tendon pulls proximalward without affecting the capsule of the metacarpophalangeal joint. The action is transmitted to the middle phalanx and to the distal phalanx by means of the lateral bands of the extensor tendon (fig. 3). However, the action of the tendon on the two distal phalanges is prevented soon

after the initiation of movement by the short fibers which unite the extensor tendon to the capsule of the metacarpophalangeal joint. The extension of the two distal phalanges is also limited by the relative shortness of the long flexors of the hand. Further pull of the common extensor is transmitted to the capsule of the metacarpophalangeal joint and to the proximal phalanx producing hyperextension of the proximal phalanx. The middle band of the extensor tendon becomes then very tense and prevents further motion from being transmitted to any part of the extensor apparatus. The two lateral bands in this position are now somewhat relaxed (fig. 4). The relative relaxation of the lateral bands is due to the arrangement of the fibers of the dorsal aponeurosis which permit slight independent motion between the middle

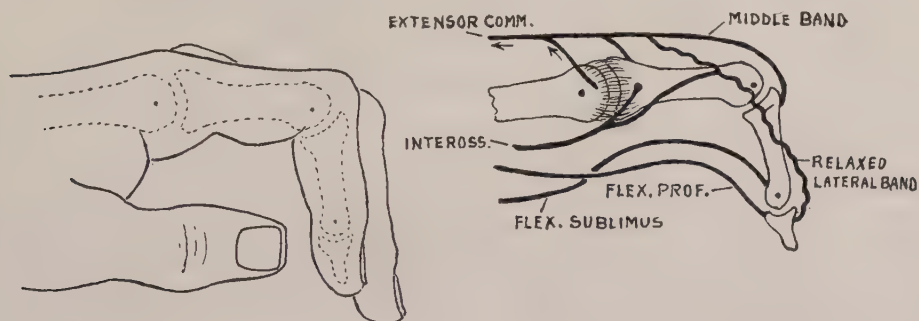


Fig. 4 Diagrammatic illustration of the finger extended at the metacarpophalangeal joint and flexed at the proximal interphalangeal joint. The pull of the common extensor tendon is almost completely utilized through the insertion to the base of the proximal phalanx, extending or hyperextending the proximal phalanx. The lateral bands are represented by a wavy black line to show that they are somewhat relaxed. The common extensor is now unable to produce extension of the distal phalanges.

and lateral bands (fig. 2) of the common extensor tendon. The intrinsic muscles acting then on the lateral bands produce extension and hyperextension of the two distal phalanges, while the proximal phalanx is hyperextended and stabilized by the short fibrous connections of the extensor communis to the capsule of the metacarpophalangeal joint (fig. 5).

If the action of the intrinsic muscles is very strong and predominates over the action of the common extensor, the proximal phalanx flexes and the two distal phalanges extend (fig. 6). The simultaneous flexion of the proximal phalanx and extension of the two distal phalanges is due to the location of the dorsal extensor apparatus which is anterior to the axis of rotation of the metacarpophalangeal joint and posterior to the axis of rotation of the proximal and distal interphalangeal joints.

Lateral motion of the fingers is produced by the action of the intrinsic muscles. When all the phalanges are extended, however, the middle finger can be moved laterally by the action of the intrinsic muscles even when the proximal phalanx is flexed to an angle of about 45° and the two distal phalanges are extended.

The lumbricals act together with the interossei and in some instances may take over the function of the interossei.

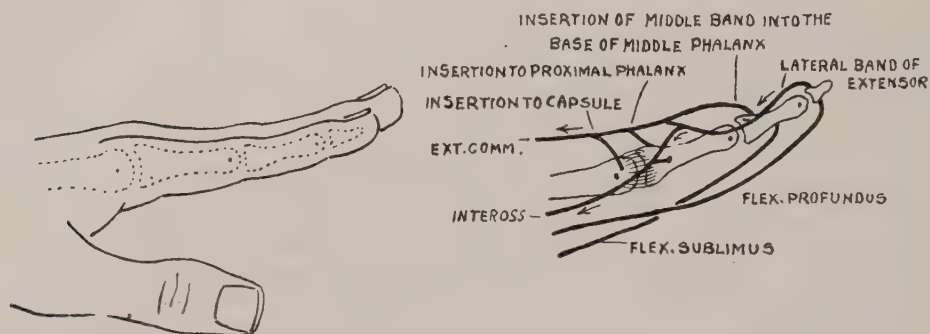


Fig. 5 Diagrammatic illustration of the finger extended in all the three joints. This action is possible by stabilization of the proximal phalanx by the common extensor tendon and extension of the two distal phalanges by the action of the interossei as shown by the small arrows.

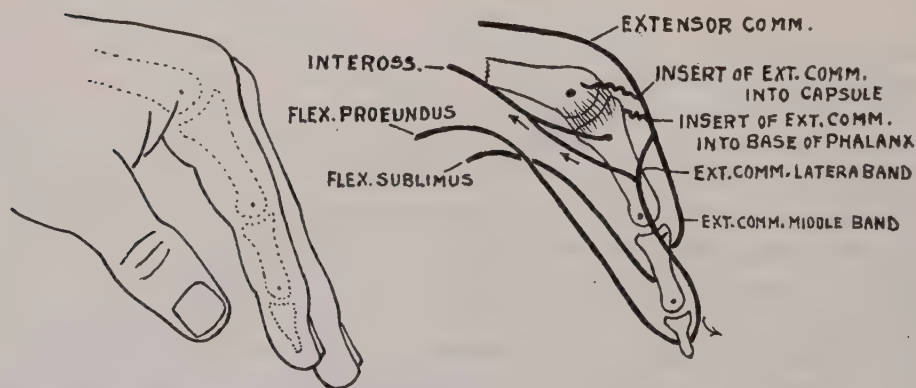


Fig. 6 Diagrammatic illustration of the finger flexed at the metacarpophalangeal joint and extended at the proximal and distal interphalangeal joints. The insertion of the extensor communis into the proximal phalanx or capsule of the metacarpophalangeal joint is shown by a wavy line indicating its relaxation. In this position the action of the interossei predominates flexing the proximal phalanx and extending the two distal phalanges. The lateral band is shown to be dorsal to the center of rotation of the interphalangeal joints.

SUMMARY

The extensor communis digitorum tendon was dissected in twenty-three fresh and embalmed human cadavers. It was found that the short insertion into the base of the proximal phalanx occurred in only 38.5% of the 140 fingers examined. Anatomical experiments showed that this insertion, contrary to accepted belief, is not essential functionally.

The action of the extensor apparatus and of the intrinsic muscles of the hand was studied by both anatomical experimentation on the cadaver material and by electrical stimulation in more than 100 living subjects. This study emphasized the importance of the connection between extensor tendon and the capsule of the metacarpophalangeal joint in the normal extension of the phalanges.

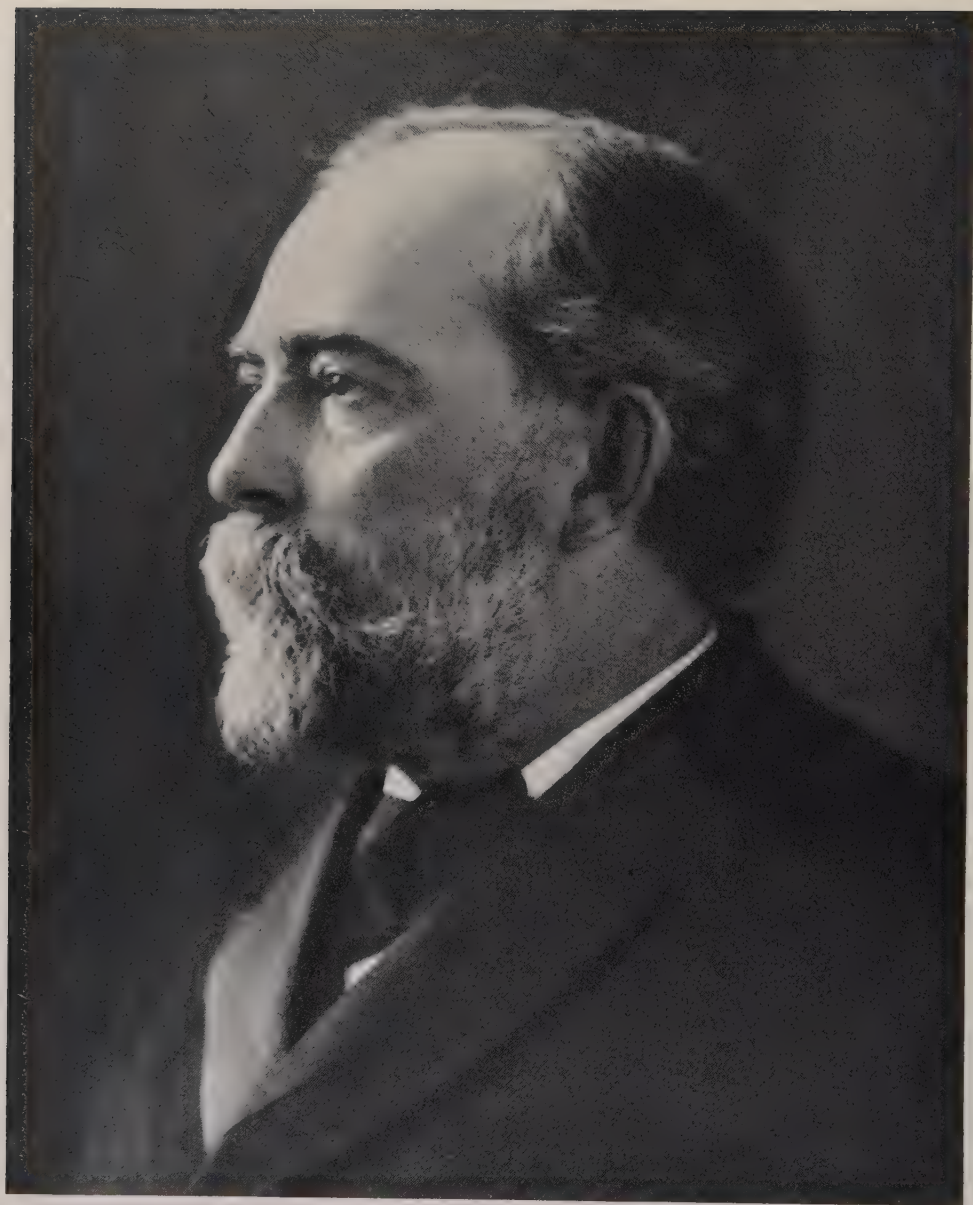
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PROCEEDINGS
OF THE
AMERICAN ASSOCIATION OF ANATOMISTS

1945

The following eight memoirs of deceased members of the American Association of Anatomists were prepared by committees appointed by Acting President J. P. Schaeffer.



Simon H. Gage

SIMON HENRY GAGE

1851-1944

In the death of Professor Gage, October 20, 1944, at his home in Interlaken, New York, the Association lost one of its oldest members and an enthusiastic supporter.

Simon Henry Gage was born May 20, 1851, on a farm in Otsego County, New York, getting his early education in the rural schools of the district. He early began an independent career and for several years was an itinerant photographer, thereby gaining experience in dealing with people and mastery of a technique which served his needs in the subsequent years. He entered Cornell University in 1873 and very soon became a student assistant in the department of zoology. He had planned to prepare himself for a medical career. He was dissuaded by his chief Dr. Burt Green Wilder, who, recognizing his abilities, urged him to devote his life to teaching and research. The department with which he became associated had, however, a distinctly "medical" atmosphere and early established a medical preparatory course.

Professor Gage soon developed an interest in anatomy, particularly microscopic anatomy. His devotion to the microscope and its use is expressed in his book, "The Microscope" which had its beginning in 1881, the last revised edition (the 17th) appearing in 1941.

Upon graduation his advance was rapid, through successive grades to professor (of Histology and Embryology). With the founding at Cornell University of the New York State Veterinary College in 1896, Professor Gage became the head of a separate department. In 1901, 3 years after the establishment of the Cornell University Medical College, of whose faculty he became a member, his department was transferred to the newly erected Stimson Hall which has been his scientific home ever since.

To his colleagues and students at Cornell there was granted a privilege less commonly available to friends away from Ithaca, the hospitality of his home, for invitations were frequent and cordial. He married December 15, 1881 Susanna Phelps who had graduated from Cornell in 1880. Mrs. Gage thoroughly shared her husband's interest in

student and science, cooperating as co-author in a number of his scientific articles and providing the illustration in others, for she was an able artist. Mrs. Gage was, however, a scientist of ability in her own right, gaining a special interest in the vertebrate brain and publishing papers of recognized merit.¹ To them was born a son, Henry Phelps Gage, who, after graduate work in physics at Cornell, joined the staff of the Corning Glass Works. There, in charge of the optical laboratory, he contributed to the perfection of several forms of special glass. Mrs. Gage died October 5, 1915. On April 14, 1933 Professor Gage and Clara Covert Starrett were married in Interlaken, New York. They were granted 11 years of happy companionship during which Professor Gage thus had devoted assistance in all phases of his many activities.

Professor Gage early joined the American Society of Microscopists of which he was twice president, and also the American Association for the Advancement of Science, twice serving as chairman of the section for zoology. In 1888 he joined the newly established Association of American Anatomists and with this organization his affiliation was most close. In 1901, through the efforts primarily of Prof. Franklin P. Mall of Johns Hopkins, Charles S. Minot of Harvard and George S. Huntington of Columbia, the American Journal of Anatomy was established. Professor Gage was chosen a member of the original board of the Journal and served for many years. When in 1905 The Wistar Institute was reorganized and became the strong support for American biology that it now is, Professor Gage was made a member of its Advisory Board. In 1931 on his eightieth birthday and in appreciation of his services, the forty-eighth volume of the American Journal of Anatomy was dedicated to him. Subsequently Professor Gage was selected by the nominating committee for President of the anatomical association, but he declined the nomination.

The references mentioned above to honors bestowed upon Professor Gage outside his own university reflect the general appreciation of the sterling qualities which characterized him as a man and a scientist and also as a teacher and counsellor of students. As a teacher he was thorough and exceptionally careful that his statements, illustrations and demonstrations were clear and pertinent. Throughout his life he exhibited for his work the enthusiasm of youth and dominant in all his efforts was a keen desire to promote research and the welfare of his fellow beings in any way he could. His advice and counsel were freely

¹ The twenty-seventh volume of the Journal of Comparative Neurology was dedicated to the Memory of Susanna Phelps Gage, and there was included a biography and a bibliography.

given to students, colleagues and fellow scientists. Doubtless many will recall his words of commendation, friendly criticism or his sympathetic discussion of a paper read at a scientific meeting, especially if it were a first paper of a young scientist. This attitude of kindly helpfulness won for him the affection of many and was early acquired. As a student assistant and after graduation in 1877 as assistant, his teaching activities brought him close friendship with many whose subsequent careers in biology or medicine proved distinguished, most notably perhaps Dr. Theobald Smith.

Perhaps the most concrete expression of appreciation was the establishment at Cornell University of a graduate fellowship in his honor. The fund for the Simon Henry Gage Fellowship in Animal Biology was first presented to the University at a dinner in honor of Professor Gage on his sixty-fifth birthday and effectively completed by the ninety-th birthday when he was again feted as a dinner guest by his friends and colleagues.

Professor Gage relinquished his teaching in 1908 to devote himself exclusively to research work—in progress or in prospect. He returned to teaching, however, in 1917–1918 when World War I depleted the Department of younger members of its staff; thus again graduate and undergraduate students could experience his clear and forceful exposition.

When in 1916 he became “Professor Emeritus” his technical retirement altered in no way his devotion to the problems in hand and he remained active in his Stimson Hall laboratory until the end of his long life. The publications of Professor Gage, books, scientific and other articles, reviews, exceed two hundred. During the early years scientific and technical papers predominate. With the broadening of his interests with the years articles of more general significance are interspersed; after his official retirement, as friends of long standing pass away, biographies and memoirs appear more frequently, but always the published results of his own scientific work. His last published paper bears the date of 1942 and at the time of his death a book was written, the manuscript for another largely completed. Aside from his interest in science, in this last epoch of his life, Professor Gage remained as always eager to help others by suggestion or with advice, or with more concrete aid.

R. R. BENSLEY

B. F. KINGSBURY

GEORGE L. STREETER

ROBERT BENNETT BEAN

1874-1944

Robert Bennett Bean, Professor of Anatomy at the University of Virginia from 1916 until 1941 when he resigned because of ill health, died in a hospital at Staunton, Virginia on August 27, 1944. He was born at "Pleasant Hill", Gala, Botelourt County, Virginia on March 24, 1874. After receiving the Bachelor of Science degree from the Virginia Polytechnic Institute in 1900, he entered the Medical School of Johns Hopkins University, graduating with the class of 1904. Subsequently he served as instructor of anatomy at Johns Hopkins for 1 year, as assistant professor of anatomy at the University of Michigan for 2 years, as associate professor of anatomy at the University of the Philippines for 3 years, and as professor of anatomy at Tulane University for 6 years.

Dr. Bean's research interests lay chiefly in the field of physical anthropology. For a number of years he offered an elective course in anthropology to senior medical students. During the period of his professorship of anatomy at the University of the Philippines from 1907 to 1910 and at Tulane University from 1910 to 1916 he was especially active in investigations concerning racial types. A large body of anthropological data was included in a book on "The Racial Anatomy of the Philippine Islanders", another on "The Races of Man", and in his most recent book entitled "The Peopling of Virginia". For many years he served as Associate Editor of the American Journal of Physical Anthropology, and in 1926 as chairman of the Anthropological Section of the American Association for the Advancement of Science. Among his more important publications are the articles on "Philippine Types", "The Pulse of Growth in Man", "Some Anatomical Characters of the Mongoloid Dwarf", and "Weights or Organs".

In the 25 years that Dr. Bean held the professorship of anatomy in the Medical School of the University of Virginia a host of students profited from his stimulating instruction. Of pleasant and genial nature, he enjoyed presenting many of the facts of gross anatomy in light humorous vein. About 15 years ago he constructed a unique model of the peritoneum. This model has been a welcome aid to teachers of anatomy. He also wrote a special treatise on the anatomy of the pelvis

and lower extremities. Some of the current textbooks of anatomy refer to his work on the teeth of children, on the subclavian artery, and on certain nerve connections in the head.

Dr. Bean is survived by his widow and four children: two daughters, Mrs. James VanDeusen Epps and Mrs. Nat Emery; and two sons, Major William Bennett Bean, Medical Corps, United States Army, and Rev. George Bean, Chaplain, United States Navy.

H. E. JORDAN

J. E. KINDRED

C. C. SPEIDEL

IRVING HARDESTY

1866-1944

Irving Hardesty was born at Beaufort, North Carolina, on October 8, 1866. He died on November 7, 1944, 11 years after retirement from the position which he had held since 1909 as professor and head of the department of anatomy in Tulane University, School of Medicine.

Hardesty graduated from Wake Forest College in 1892; in 1918 Wake Forest conferred upon him an honorary degree, the Doctor of Science. Hubert A. Royster, his college mate and friend, describes him in the undergraduate period as "a keen student and an assiduous worker", and adds: "I do not know of anyone who possessed a sounder scientific attitude or who had a finer appreciation of the principles of biology. An ardent pupil of the late Dr. William L. Poteat, Hardesty was already head and shoulders above the rest of his classmates in scientific subjects."

The year following graduation was spent as principal of Wakefield Academy, in North Carolina, after which (1895-1896) he served as assistant in biology at the University of Missouri. It was at the University of Chicago that his advanced training was obtained, mainly under Henry H. Donaldson whose assistant he was until 1900. His was the first Ph.D. degree in the biological sciences to be granted by that institution (1899). In 1900 Hardesty joined the anatomy staff at the University of California as instructor; he was soon advanced to the rank of assistant professor and then to an associate professorship, which he resigned in 1909 to assume charge of the department at Tulane.

Hardesty's chief investigations concerned neuroglia, the tectorial membrane and the composition of peripheral nerves. Several editions of Morris' Human Anatomy carried his section on the nervous system. His Neurological Technique (1902) was a distinct contribution toward advance of neurological teaching and research. A Laboratory Guide for Histology (1908) embodied his conceptions of the mechanics of proper teaching of histology. His basic philosophy of anatomical teaching was set forth in a description of the department of anatomy at Tulane, published in *Methods and Problems of Medical Education* (1930).

Memberships in scientific societies included the American Association of Anatomists, in which he was elected for three terms as member of the Executive Committee, the American Association for the Advancement of Science (fellow, 1914-), Society for Experimental Biology and Medicine, Society of Endocrinologists, and New Orleans Academy of Science (president, 1923-1930). In 1909 Hardesty entered upon a long service as member of the Editorial Board of the *Anatomical Record*. In the early 1920's he shared in the work of the National Research Council, taking active part in the committee concerned with investigations of the vestibular division of the inner ear. During World War I he acted as liaison officer between the University and the administration of Camp Martin, the Tulane unit of the Students' Army Training Corps. His counsel was sought in many affairs of the University, to which he gave generously of his time and effort through service on numerous committees.

Many years ago Hardesty began the writing of a text-book of histology. The illustrations were largely completed, but the manuscript was laid aside unfinished. He abandoned it with an explanation characteristic of his perfectionist ideals, self-distrust for a venture into the welter of conflicting opinions on certain phases of hemopoiesis. For years there hung over his desk the motto, "He who thinks a perfect work to see thinks what n'ere was nor ere shall be." Hardesty not only strived to approach the perfect in his own undertakings but constantly held to it as the goal toward which he led students and colleagues. His emphasis on standards was vigorous and unremitting. Hardesty's associates will ever remember that staunch devotion to high standards — in science, in teaching, in conduct of the department and institution, and in personal honor.

WILBUR C. SMITH
HAROLD CUMMINS
RUDOLPH MATAS

GENE WALKER HAIR

1908-1944

Gene Walker Hair, Instructor of Anatomy at the University of Buffalo School of Medicine, was killed in action June 9, 1944, when a Landing Ship Tank was torpedoed off the coast of France. He had been in North Africa, Sicily, Salerno and England. He is survived by his wife and son.

Dr. Hair was born in Tekoa, Washington, August 4, 1908. He received the B.S. and M.S. degrees from the State College of Washington in 1932 and 1934 respectively. He joined the staff of the Department of Anatomy of the University of Buffalo and worked for his doctorate under the supervision of the late Dr. Wayne J. Atwell. In 1937 he completed his work for the Ph.D. degree and wrote his thesis on "Study of Certain Details of the Mammalian Hypophysis Cerebri: I. Blood Supply. II. Nerve Supply. III. Cytology of the Pars Tuberalis." He continued to teach in the Department of Anatomy while studying medical subjects. Dr. Hair received his M.D. degree in 1941. After completing his internship at the Sisters of Charity Hospital, he practiced medicine and continued as instructor of anatomy until he was commissioned a lieutenant (jg) in the Medical Corps of the U. S. Naval Reserve on March 12, 1943.

Dr. Hair was a member of the American Association of Anatomists, the Alpha Omega Alpha Honorary Medical Fraternity, Sigma Xi, American Association for the Advancement of Science, and the James A. Gibson Anatomical Society of the University of Buffalo.

Dr. Hair was a capable and energetic investigator. His genial disposition made him able to cooperate well with his associates and made him well liked by the students. The war has taken another promising young man from our midst.

OLIVER P. JONES
R. R. HUMPHREY
F. E. EMERY

WALTER HUGHSON

1891-1944

Walter Hughson was born at East Orange, New Jersey, on August 4, 1891, and was a graduate of Princeton (B.S., '14) and of Johns Hopkins (M.D., '18). Throughout his career the problems that interested him most were those which combine clinical aspects and the basic sciences. A 2-year medical internship and residency at the Union Protestant Infirmary (the predecessor of the Union Memorial Hospital) in Baltimore was followed by 2 years (1920-1922) on the full-time staff of the Department of Anatomy at Hopkins, and this in turn by a period during which he was a member of the staff of the Department of Surgery but gave the course in Applied Anatomy and carried on investigations in the Anatomical Laboratories. To the members of this Association the best known of the studies of this period of his life are those on the cerebrospinal fluid, with L. H. Weed, and on the innervation of facial muscles, with Ernst Huber. In 1926 Dr. Hughson opened a private office for the practice of surgery and became a visiting, instead of a full-time, member of the staff at Hopkins. In 1930, however, because his interest in otology had been roused by a severe illness that left one ear totally deaf, Dr. Hughson closed his office for the practice of surgery and attacked problems of deafness. From this time until his death 14 years later he devoted himself to the study of hearing, particularly the causes of impaired hearing and what could be done to help the victims of this affliction to become more useful and happy members of society. His studies in this field covered a wide range and included topics seemingly as diverse as the effect of experimental lesions on the cochlear potentials of animals and the effectiveness of different methods of teaching deaf children to speak; always, however, they contributed to the solution of the central problem. The first few years of this period of his life were spent in the otolaryngological department at Hopkins, the last decade of it was in the Otological Research Laboratory founded for him and directed by him at the Abington Memorial Hospital near Philadelphia.

Pneumococcic meningitis, combined with a series of circumstances as tragic as those that frequently prevented the recovery of soldiers wounded in combat, caused Dr. Hughson's death on September 13, 1944.

Until 2 days previously, he was working long hours in his special laboratory fitting deafened Naval personnel with hearing aids, applying methods he himself had developed and gathering data on which to base further advances in this complex field of research. The zeal and enthusiasm with which he threw all his energies into this, the latest major investigation he undertook, was characteristic of the way he attacked other important problems that intrigued his interest. Dr. Hughson's death, therefore, was as definitely a direct loss to the war effort of our nation as though he had actually been in the uniform of his country.

Dr. Hughson was elected to membership in the American Association of Anatomists in 1921, and although in recent years but few of his investigations fell in the category of topics suitable for our programs, he retained his interest in the work presented by others and often attended the meetings.

Dr. Hughson's family life was in the highest American tradition, and it was at times hard to say whether his enthusiasm for his family — wife, two daughters and two sons — or for his scientific studies was the greater. To the members of his family, all of whom survive him, the American Association of Anatomists wishes formally to express its sympathy and its sense of loss at this time.

STACY R. GUILD
GEORGE B. WISLOCKI

HENRY CUSHIER RAVEN

1889-1944

Henry Cushier Raven, Curator of the Department of Comparative Anatomy of the American Museum of Natural History and a member of the American Association of Anatomists, died on April 5, 1944. Mr. Raven combined in a very exceptional degree the careers of naturalist-explorer, museum curator and comparative anatomist.

As a boy in Bay Shore, Long Island, he early showed a preference for outdoor life and natural history. After preliminary training in taxidermy and collecting, he was sent at the age of 23 to the Netherlands East Indies to collect mammals and birds and other vertebrates for

the Smithsonian Institution. He spent 6 years collecting in Sumatra, Celebes, Borneo and adjacent regions, and later in East and South Africa. In 1919 he joined the American Museum of Natural History and thenceforth made extensive collections for that Museum in Australia, Africa, Malaya, Burma, Greenland. In 1939 he again collected in Australia and in 1940 he visited Peru and Ecuador. During these expeditions he secured an enormous amount of valuable material, not only skins and skulls but carefully injected anatomical specimens, and thousands of photographs and field records.

In the intervals between his expeditions, Mr. Raven acquired an expert knowledge and facility in the anatomy of man and other vertebrates, serving for several seasons as Instructor and Demonstrator in Anatomy at the Johns Hopkins University, and preparing many excellent dissections for his projected monograph on the anatomy of the gorilla. The material for this monograph was collected by him on an expedition sent by Columbia University and the American Museum to the Belgian Congo and the French Cameroons. About three-quarters of the work was completed at the time of his death and the remaining parts are being prepared by his friend and colleague, Dr. John Eric Hill of the American Museum of Natural History. It is planned to issue this and other papers dealing with the anatomy of the gorilla in the Henry Cushier Raven Memorial Volume.

Raven was the author of many significant papers dealing with such topics as the comparative anatomy and classification of the kangaroos, the giant panda, the sperm whale and other cetaceans, the anatomy and evolution of the Ocean Sunfish, *Mola mola*; his analysis of the mammalian faunas on either side of Wallace's Line contributed to a better understanding of the nature and geologic history of that famous boundary between the Malaysian and Australian regions.

His very numerous friends and colleagues were strongly drawn to him, partly because of his life-long habit of accuracy and objectivity in the study of living animals; partly for his ability to recreate his illuminating observations in the field; and not least for his friendly and helpful relations with human beings in all climates and stations of life.

WILLIAM K. GREGORY
FRANZ WEIDENREICH
JOHN E. HILL

HENRY WILSON STILES

1875-1944

Born in Savannah, Missouri, March 6, 1875, of a family of Virginia pioneers; he attended Peekskill Military Academy, then matriculated at the College of Physicians and Surgeons when he first focused his interest on Human Anatomy. (Exact date unknown.)

His medical diploma was received in 1901 from Ensworth Central Medical College after his marriage to Reba Cole of St. Louis. His two talented daughters were born during his few years of practice near Stamford, Col. About 1905 he returned to Columbia, Mo., and became Instructor in Anatomy. From 1906-09 he was Instructor at Michigan under J. P. McMurrich and as a colleague of G. E. Streeter and McCotter. Next he spent a year at Tulane as Assistant Professor under Hardesty. From there he moved to Syracuse to replace H. D. Senior as Professor of Anatomy. Routine anatomical instruction prospered during his administration.

Dr. Stiles was loved by associates and students for his personal charm, and admired for the artistic perfection of his anatomical demonstrations. He taught by the example method; a skilful display of anatomical relations was executed at each dissecting table under the eyes of watching students and their imitative response was a joy to watch. For regional reviews he prepared many personal dissections, which were demonstrated and used for small-group examination. Many of these are still in use in the Syracuse Laboratory.

During his vacations he made many trips to Europe where he culled much technical information at the leading museums. Two hobbies shared his free time, stereoscopic photography of anatomical specimens, and the collecting and restoring of early American furniture. His zeal was great in pursuit of his duties and these hobbies. After the marriage of his daughters (during the war years) he focused almost all of his time and efforts upon teaching and his hobbies, even to the detriment of his health, and was heldom seen at meetings of the Association.

Dr. Stiles retired in June 1936 after several hospital episodes; his wife died and he soon removed to make his home in Scarsdale with his

daughters. Here he continued both his hobbies until his death September 5, 1944. That he was unable to complete and publish his exquisite stereoscopic plates, as a memorial to his industry, remains the regret of all who were associated with him.

PHILIP B. ARMSTRONG

WALTER F. GREENE

THEODORE SNOOK

CONRAD ENGERUD THARALDSEN

1884-1944

On May 20, 1944, Conrad Engerud Tharaldsen, Professor of Anatomy and Head of the Department at New York Medical College, died. It was his sixtieth birthday. His heart, which had first warned him sharply in 1936, finally gave up a losing battle.

He was born at Battle Lake, Minnesota, where his father was the Lutheran pastor of pioneer Norwegians who contributed their share of living to Rølvaag's *Giants in the Earth*. In the last years of Conrad Tharaldsen's life, those of us who watched his grim wrestling were ever reminded of those giant Scandinavians and of their ancestors in turn, who were giants on the sea.

Young Tharaldsen received his B.S. at St. Olaf College in 1907. Some post graduate work at Harvard was followed by 8 years as a teacher of biology in Blain High School, Superior, Wis., and in the State Normal School at Mayville, N. Dak. He moved on to the M.A. degree at Columbia University in 1918, and thereupon was appointed assistant professor of Zoology at Northwestern University. He maintained his Columbia association during this time; so that in 1925 the University awarded Professor Tharaldsen its Ph.D. degree in cytology. He had had the high privilege of studying under a galaxy of the great, Morgan, Conklin, Gregory, et. al. In 1924 he had been promoted at Northwestern University to associate professor. Shortly afterward, on the sudden death of Professor Locy, he became the acting head of the Dept. of Zoology. In 1927 he was invited to the headship of the Dept. of Anatomy, New York Medical College. The invitation came on the eve of a

new period of rapid development and expansion in this institution, which was to include its absorption of some more hospitals and acquisition of further clinical affiliations with the hospital system of metropolitan New York. Dr. Tharaldsen's cytological experience had been leading him to experiments with ceanothyn and exploration in the cytological phenomena of cancer. At first, after coming here, he attempted to continue these interests actively; but arriving as he did at a time when much administrative timber had to be felled and hewn, he plunged into this kind of pioneering. He was eminently suited for it. The school is very aware of what it owes him in the matter. The best monument that has risen to him is a Department of Anatomy far superior to anything the college has had before. It is also a tribute to him that he saw it named the William Waldo Blackman Laboratory of Anatomy, in honor of one of the school's grand old men of anatomy and medicine; and that while Dr. Blackman, emeritus professor of anatomy, was still in status to be aware of it. It remains de facto a monument to Professor Tharaldsen quite as well.

Professor Tharaldsen was not among those who forget the vital role of the schoolmaster in science. Gradually, indeed, the schoolmaster had to force the scientific investigator from the floor. But only from the floor. If the task of teaching others crowded upon that of original exploration, he still would encourage in others the activity for which his own time seemed to be vanishing. At last that nemesis, all too familiar to his ilk, smote his sturdy Norse constitution. It could not lay the Viking. He would live as long as there was living to do, even if on borrowed time. Schoolmaster and departmental administrator he would remain; but in addition, he launched upon a new and (as those of us who saw the work can testify) unique Atlas of Human Anatomy.

Generals nowadays die with their boots on. Conrad Tharaldsen had been seeing to the planting of new onion sets in his Victory Garden when, this time, it was death itself that changed his career.

EARL W. COUNT

CHARLES A. WOERNER

JAMES W. BENJAMIN

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

1945

During the summer of 1944 prospects for a regular meeting in 1945 looked so bright that the Executive Committee voted in favor of planning a meeting to be held in Cleveland, at the invitation of Western Reserve University, May 2-4. All early arrangements had been made, and the material for notice of meeting prepared for the printer, when suddenly, early in January, transportation difficulties again increased to such an extent that it was necessary for the third year in succession, to cancel the meeting. It was decided again, as in the two preceding years, to carry on all feasible activities of the Association — printing of "Abstracts" and "Proceedings", nomination and election, to provisional membership, of new members, and authorization for the holding of strictly local meetings. All have been carried out.

In expectation of the holding of a 1945 meeting, the Nominating Committee, which has been continued since its appointment in 1942, was requested by the Executive Committee to prepare nominations for the several offices. The nominees are as follows: for President, George W. Corner; for First Vice-President, Edward Allen Boyden; for Second Vice-President, Marion Hines — each for a term of 2 years; for Secretary-Treasurer, Normand L. Hoerr — for a 4-year term; for members of the Executive Committee for 4 years, William Bloom and William W. Greulich (the last two having been originally nominated in 1943). With the cancellation of the meeting, action upon the nominees was postponed in accordance with an earlier ruling by the Executive Committee that "these names will be presented whenever the next annual meeting may be held — the time between the 1942 meeting and that date being considered an interim period, counting as 1 year, with respect to tenure of officers and members of the Executive Committee".

Reports on finances, membership, etc. In May, 1945, the Secretary-Treasurer sent a report to the members of the Executive Committee with a request for a vote on the following eight items. They are largely matters customarily acted upon at the annual business session of the Association, following action by the Executive Committee, but for which the Association has delegated to the Executive Committee power to act during the "interim period":

1. (a) *Financial statement for the year 1944*

Balance on hand in checking account December 31, 1943, in First National Bank, Philadelphia, Pennsylvania	\$1,916.58	
Receipts from dues	979.92	
Total credits		\$2,896.50

Expenditures

Postage, stationery, supplies and miscellaneous	95.48	
Wistar Institute		
Abstracts and postage	401.40	
Proceedings and postage	174.91	
Printing	30.75	
First National Bank		
Returned checks	3.00	
Charges for handling account	8.75	
Total expenditures		714.29
		\$2,182.21

Assets

Balance on hand December 31, 1944 and deposited in the name of the American Association of Anatomists in the First National Bank, Philadelphia, Pennsylvania	\$2,182.21	
War Bond Defense Series F dated May, 1942 in safe deposit box, First National Bank, Philadelphia, Pennsylvania		
Value December, 1943	\$750.00	
Increase in value of war bond in 1944	10.00	760.00
Total assets		\$2,942.21

Total assets increased by \$275.63 during the year, resulting from increase of \$265.63 in the general account and \$10.00 in the value of the war bond.

1. (b) Acting President Schaeffer appointed an Auditing Committee consisting of W. H. F. Addison, Chairman and E. J. Farris, who submitted the following report:

May 9, 1945

The undersigned Auditing Committee has examined the accounts of the Secretary-Treasurer for the year 1944 and has found total receipts reported to be those entered in the journal, expenditures in accordance with receipted bills and the balance on hand Dec. 31, 1944 in agreement with the bank statement of that date.

Signed:

W. H. F. ADDISON
E. J. FARRIS

The Executive Committee voted to accept the reports of the Treasurer and of the Auditing Committee.

2. It was recommended that for 1945 the dues be continued at \$1.50, with the explanation that this handles cost of "Abstracts" and "Proceedings" for all members, takes care of non-paying members, and,

with the surplus of the past two years, will cover expenses of the 1946 meeting.

Adopted by the Executive Committee.

3. It was reported that two members had sent their resignations. The Executive Committee was reluctant to accept them, and this reluctance has been communicated to the two members by the Secretary. One of the two, A. Brazier Howell, prefers that the resignation stand. Action on the second has been postponed.

4. It was reported that the Association had suffered great loss during the past year through the death of eight members; that Acting President Schaeffer had appointed committees to prepare memorials to each of the eight, and that they, with the following brief notices, would be printed in the Proceedings, if approved by the Executive Committee. With the endorsement of the Committee, the memorials are included at the beginning of these "Proceedings", and the brief notices are as follows:

ROBERT BENNETT BEAN, born March 24, 1874, died August 27, 1944. Elected to membership in 1904. Professor of Anatomy, University of Virginia.

SIMON HENRY GAGE, born May 20, 1851, died October 20, 1944. A member of the Association from its first year, 1888. Member Executive Committee 1906-1911. Emeritus Professor of Histology and Embryology, Cornell University, Ithaca.

GENE WALKER HAIR, born August 4, 1908, died June 9, 1944. Elected to membership in 1939. Instructor in Anatomy, University of Buffalo. Killed in action off the coast of France.

IRVING HARDESTY, born October 8, 1866, died November 7, 1944. Elected to membership in 1901. Member Executive Committee 1910, 1912-1915, 1922-1923. Emeritus Professor and Head of Department of Anatomy, Tulane University.

WALTER HUGHSON, born August 4, 1891, died September 13, 1944. Elected to membership in 1921. Director Otological Research Laboratory, Abington Memorial Hospital, Abington, Pennsylvania.

HENRY CUSHIER RAVEN, born April 16, 1889, died April 5, 1944. Elected to membership in 1928. Curator, Department of Comparative Anatomy, American Museum of Natural History.

HENRY WILSON STILES, born March 6, 1875, died September 5, 1944. Elected to membership in 1906. Professor of Anatomy, Syracuse University.

CONRAD ENGERUD THARALDSEN, born May 20, 1884, died May 20, 1944. Elected to membership in 1933. Professor of Anatomy and Chairman of the Department, New York Medical College.

5. Report on nominees for membership. The names of thirty-three nominees were sent to the members of the Executive Committee. Four were questioned and they, together with two questioned by the Secretary and three renominations are held until the next meeting of the Executive Committee, when their qualifications can be discussed. The twenty-nine nominees endorsed by the Executive Committee will be considered as *Provisional Members* until the next annual meeting, before which time they cannot be voted upon in accordance with Article V, Section 1, of the Constitution of the Association. The names of the new *Provisional Members* are as follows, the names in parentheses being those of the sponsors:

- ATKINSON, WILLIAM BROCKLISS, B.S., M.S., Ph.D., Instr. Anat., Columbia. (S. R. Detwiler, P. E. Smith.)
- BLANDAU, RICHARD J., A.B., Ph.D., Instr. Anat., Rochester. (C. E. Tobin, K. E. Mason.)
- CARPENTER, ESTHER, B.A., M.S., Ph.D., Assoc. Prof. Zoology, Smith College. (J. S. Nicholas, E. A. Scharrer.)
- DEANE, HELEN WENDLER, B.A., M.A., Ph.D., Instr. Anat., Harvard. (G. B. Wislocki, E. W. Dempsey.)
- DE BRUYN, PETER P. H., M.D., Asst. Prof. Anat., Chicago. (G. W. Bartelmez, W. Bloom.)
- ELIAS, HANS MICHAEL, Ph.D., Prof. Hist., Middlesex School of Vet. Med. (F. T. Lewis, H. L. Weatherford.)
- FINERTY, JOHN C., A.B., M.S., Ph.D., Instr. Anat., Michigan. (B. M. Patten, R. E. McCotter.)
- GOREMAN, AUBREY, A.B., M.S., Ph.D., Res. Fellow Anat., Yale. (W. U. Gardner, C. A. Pfeiffer.)
- HARTMANN, J. FRANCIS, A.B., Ph.D., Instr. Anat., Albany. (J. M. Wolfe, J. L. Schwind.)
- HOUSE, EARL LAWRENCE, B.S., M.A., Ph.D., Asst. Prof. Anat., Emory. (H. Blincoe, J. H. Venable.)
- JUHN, MARY, Ph.D., Res. Assoc. Prof., U. of Maryland. (B. H. Willier, M. E. Rawles.)
- KIRGIS, HOMER D., M.D., Ph.D., Instr. Neuroanat., Tulane. (H. Cummins, A. F. Reed.)
- KONEFF, ALEXEI ALEXANDER, M.D., Asst. Prof. Anat. and Lecturer in Hist. Tech. California. (H. M. Evans, M. E. Simpson.)
- LIEDKE, KATHE B., M.Sc., Ph.D., Instructor, N. Y. Med Coll. (M. B. Stark, J. W. Benjamin.)
- MACKLIN, MADGE THURLOW, A.B., M.D., LL.D., Asst. Prof. Emb. and Hist., Western Ontario. (C. C. Macklin, E. R. Clark.)
- MCCULLOCH, WARREN S., A.B., A.M., M.D., Assoc. Prof. Psychiatry, Illinois. (H. W. Magoun, G. von Bonin.)
- MCCULLOUGH, ALBERT WILLIAM, A.B., M.A., Ph.D., Asst. Prof. Anat., Arkansas. (B. L. Robinson, D. A. Rhinehart.)
- MORGAN, CHARLES F., A.B., Ph.D., Asst. Prof. Pharmac. and Mat. Med., Georgetown. (O. Solnitzky, J. C. Plagge.)
- MOYER, ELIZABETH K., A.B., M.A., Ph.D., Instr. Anat., Temple. (J. F. Huber, S. R. Detwiler.)
- NICOLL, PAUL ANDREW, A.B., Ph.D., Asst. Prof. Physiology, Indiana. (R. L. Webb, R. L. Janes.)
- PALAY, SANFORD L., M.D., Asst. Res., Univ. Hosp., Western Reserve. (N. L. Hoerr, E. A. Scharrer.)

- RYERSON, DWIGHT L., B.A., M.S., Ph.D., Instr. Anat., Arkansas. (K. S. Chouke, B. L. Robinson.)
 SACCOMANNO, GENO, B.A., M.A., Ph.D., Instr. Anat., St. Louis Univ. (A. Kuntz, D. M. Schoemaker.)
 SZEPSSENWOL, J., M.D., Knight fellow, Yale. (H. S. Burr, R. G. Meader.)
 TELFORD, IRA ROCKWOOD, A.B., M.A., Ph.D., Asst. Prof. Anat., George Washington. (C. M. MacFall, G. B. Jenkins.)
 THOMAS, THURLO B., S.B., M.A., Ph.D., Asst. Prof. Anat., Texas. (C. M. Pomerat, I. H. Blount.)
 TRUSCOTT, BASIL LIONEL, B.A., M.A., M.S., Ph.D., Instr. Anat., Georgetown. (O. Solnitzky, J. M. Odiorne.)
 WHEATLEY, MAX D., M.A., Ph.D., Asst. Prof. Anat., South Dakota. (W. R. Ingram, H. Dawson.)
 WIMSATT, WILLIAM ABELL, A.B., Ph.D., Instr. Anat., Harvard. (H. B. Adelman, F. T. Lewis.)

Number of members:

Number of members May 1, 1944	790
Deceased 8	
Resigned 1	9
	781
New provisional members	
endorsed May, 1945	29
Numbers of members May 20, 1945	810

6. The question of an annual meeting in 1946. Dr. N. L. Hoerr has extended to 1946 the invitation to meet in Cleveland. In spite of uncertainties, the Executive Committee voted to make tentative preparations for a regular meeting to be held in Cleveland either on the 3 days preceding Easter, April 18-20, or on nearby dates — whichever is most convenient or available.

7. The Executive Committee endorsed the continuation of present officers, committee and delegates.

8. The Secretary reported to the Executive Committee regarding the "Anatomical Journal Trust Fund" as follows:

"The Trustees of the 'Anatomical Journal Trust Fund,' a fund established originally for the purpose of publishing a journal devoted to original investigations in Anatomy and allied science, and which is now held for 'the assistance of the publication of original investigations of anatomy and allied science, and for the publishing of the American Journal of Anatomy and Anatomical Record, should the same be turned back by the Wistar Institute to the Trustees', have offered to turn the fund over to the American Association of Anatomists, providing the fund and its income be used for the purposes mentioned. In order to hold such a fund it would be necessary for the Association to incorporate. Obviously the decision to incorporate and to accept the trusteeship of the fund, which totals about \$13,000, must await action by the Association in an annual meeting. However, certain preliminary studies might be made regarding incorporation which might save time in case the Association decides in its favor."

The Executive Committee approved a preliminary study of matters relating to incorporation of the Association.

Local Meeting — A letter received by the Secretary from Dr. L. B. Arey, Chicago, dated June 2, 1945 contained the following information: "Last week we held a local anatomical meeting at Loyola University School of Medicine. There was a joint program in the morning, two sections in the afternoon, a late afternoon showing of movies and viewing of demonstrations, and a dinner with a special address. I should estimate that 60 to 70 were in attendance; there were about 45 at the dinner".

No other local meetings were held.

ELIOT R. CLARK,
Secretary-Treasurer

AMERICAN ASSOCIATION OF ANATOMISTS

OFFICERS AND LIST OF MEMBERS

Officers

<i>Acting President</i>	Jacob Parsons Schaeffer
<i>Vice-President</i>	Leslie B. Arey
<i>Secretary-Treasurer</i>	Eliot R. Clark

Executive Committee

For term expiring 1943 (continued)	Stacy R. Guild, Edward A. Boyden
For term expiring 1944 (continued)	Joseph C. Hinsey, Carl C. Speidel
For term expiring 1945 (continued)	E. Horne Craigie, Walter E. Sullivan
For term expiring 1946	Sam L. Clark, S. I. Kornhauser

Delegates to the Council of A.A.A.S. for 1944 (continued)

Joseph C. Hinsey	W. W. Greulich
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Representative to the National Research Council 1943-1946

George L. Streeter

Member of the Commission on Standardization of Biological Stains

William F. Windle

Delegates of Union of Biological Societies

Stacy R. Guild	Normand L. Hoerr
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Committee on Nominations for 1943 (continued)

Harold Cummins, *Chairman*

Karl E. Mason	William F. Windle
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Committee on Anatomical Nomenclature

George W. Corner, *Chairman*

Committee on National Defense

J. S. Nicholas, *Chairman*

The Acting President and Secretary-Treasurer, *ex officio*

Motion Picture Committee

E. R. Clark, *Chairman*

Warren H. Lewis	Carl C. Speidel
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MEMBERS

* Indicates members in national service.

- ABELL, RICHARD G., M.A., Ph.D., Assistant Professor of Anatomy, *University of Pennsylvania School of Medicine, Philadelphia 4, Pa.*
- ABERCROMBIE, WARREN F., A.B., Sc.M., Ph.D., Head of Biology Department, *Erskine College, Due West, S. C.**
- ABERLE, (MRS.) SOPHIE D., Ph.D., M.D., *National Research Council, 2101 Constitution Ave., Washington, D. C.*
- ADAMS, A. ELIZABETH, A.B., A.M., Ph.D., Professor of Zoology, *Mount Holyoke College, South Hadley, Mass.*
- ADAMS, L. A., A.B., A.M., Ph.D., Professor of Zoology, Curator of Museum of Natural History, *University of Illinois, 401 Vermont St., Urbana, Ill.*
- ADAMSTONE, F. B., B.A., M.A., Ph.D., Associate Professor of Zoology, *345 Natural History Bldg., University of Illinois, Urbana, Ill.*
- ADDISON, WILLIAM HENRY FITZGERALD, B.A., M.D., Professor of Normal Histology and Embryology, *School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*
- ADELMANN, HOWARD B., B.A., M.A., Ph.D., Professor of Histology and Embryology, *Cornell University, Stimson Hall, Ithaca, N. Y.*
- ADES, HARLOW W., B.S., M.S., Ph.D., Assistant Professor of Anatomy, *Box 734, Emory University, Emory, Ga.*
- ALDEN, ROLAND H., B.A., Ph.D., Assistant Professor of Anatomy, *University of Tennessee College of Medicine, Memphis, Tenn.*
- ALEXANDER, WILLIAM F., B.S., M.S., Ph.D., Senior Instructor, Dept. of Microanatomy, *School of Medicine, St. Louis University, 1402 S. Grand Ave., St. Louis, Mo.*
- ALGIRE, GLENN H., M.D., Assistant Surgeon (R), U.S.P.H.S., *National Cancer Institute, Bethesda, Md.*
- ALLEN, BENNET MILLS, Ph.D., Professor of Zoology, *University of California at Los Angeles, 909 Micheltorena St., Los Angeles, Calif.*
- ALLEN, EZRA, A.M., Ph.D., Retired. Visiting Professor, *J. B. Stetson University, 237 W. Stetson St., De Land, Fla.*
- ALLEN, LANE, Ph.D., M.D., Professor of Anatomy, *University of Georgia, School of Medicine, Augusta, Ga.*
- ALLEN, WILLARD M., B.S., M.S., M.D., *Washington University, School of Medicine, St. Louis, Mo.*
- ALLEN, WILLIAM F., A.M., Ph.D., Head of Department of Anatomy, *University of Oregon Medical School, Portland, Ore.*
- ALLIS, EDWARD PHELPS, JR., C.E., M.D., LL.D. (Charter member), *Palais de Carnolès, Menton (A.M.), France.*
- ALTSCHUL, RUDOLF, M.D., Associate Professor in Histology and Neurology, *University of Saskatchewan, Saskatoon, Canada.*
- ANDERSEN, DOROTHY H., Associate in Pathology, *Columbia University; Assistant Pathologist, Babies' Hospital, 167th St. and Broadway, New York City.*
- ANDREW, WARREN, M.S., M.D., Ph.D., Associate Professor of Histology and Embryology, *Department of Anatomy, Southwestern Medical College, Dallas, Tex.*
- ANGEL, JOHN L., A.B., Ph.D., Associate in Anatomy and Physical Anthropology, *Jefferson Medical College, Daniel Baugh Institute of Anatomy, 307 S. Eleventh St., Philadelphia, Pa.*
- ANGULO Y GONZALEZ, ARMANDO W., B.L., B.S., A.B., A.M., Ph.D., *418 Beverly Blvd., Upper Darby, Pa.*

- ANSON, BARRY JOSEPH, A.B., A.M., Ph.D., Professor of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*
- APGAR, BESSIE D., Ph.D., *R.D. No. 3, Harrisburg, Pa.*
- APGAR, CHARLES S., JR., Ph.D., *R.D. No. 3, Harrisburg, Pa.*
- APPLEBY, J. I., A.B., M.B., A.M., M.D., Physician and Surgeon, Company Surgeon, N. Y. C. & St. L. R. R. Co., *105 1/2 East Main St., Bellevue, O.*
- AREY, LESLIE B., Ph.D., Sc.D., (Ex Com. '30-'34, Second Vice-Pres. '42-), Robert Laughlin Rea Professor of Anatomy, and Chairman of the Division of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*
- ARMSTRONG, PHILIP BROWNELL, B.S., M.D., Professor of Anatomy, *College of Medicine, Syracuse University, Syracuse, N. Y.*
- ASDELL, SYDNEY ARTHUR, M.A., Ph.D., Professor of Animal Physiology, State College of Agriculture, *Cornell University, Ithaca, N. Y.*
- ATKINSON, WILLIAM BROCKLISS, B.S., M.S., Ph.D., Instructor in Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York 32, N. Y.*
- ATTERBURY, RUTH RAND, A.M., Ph.D., *R.F.D. 1, Roslyn, L. I., N. Y.*
- BADERTSCHER, JACOB A., Ph.D., Professor of Anatomy, *Indiana University School of Medicine, 312 South Fess Ave., Bloomington, Ind.*
- BAGLEY, CHARLES, JR., M.D., Professor of Neurosurgery, University of Maryland Medical School; Associate in Experimental Neurology, *Johns Hopkins University Medical School, The Latrobe Apts., Baltimore, Md.*
- BAILEY, PERCIVAL, M.D., Ph.D., Professor of Neurology and Neurological Surgery, *University of Illinois, 912 South Wood St., Chicago, Ill.*
- BAILEY, RALPH JORDAN, B.S., M.S., Ph.D.*
- BAILLIFF, R. N., B.A., M.A., Ph.D., Assistant Professor of Anatomy, *Louisiana State University Medical Center, New Orleans 13, La.*
- BAITSSELL, GEORGE ALFRED, B.S., M.A., Ph.D., Professor of Biology, Yale University, *Osborn Zoological Laboratory, New Haven, Conn.*
- BAKER, BURTON LOWELL, M.S., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.*
- BAKER, DAN DYSART, A.B., M.A., M.D., Orthopaedic Surgery, *3411 Prytania St., New Orleans 15, La.*
- BAKER, R. C., A.B., M.A., Ph.D., Professor of Anatomy, *Department of Anatomy, Hamilton Hall, Ohio State University, Columbus, O.*
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- BARDEN, ROBERT BRADSHAW, A.B., Ph.D., Instructor in Anatomy, *University of Southern California, School of Medicine, Los Angeles 7, Calif.*
- BARNARD, JOHN W., M.S., Ph.D., Assistant Professor of Anatomy, *School of Medicine, University of Oklahoma, Oklahoma City, Okla.*
- BARRETT, W. C., JR., Ph.D., Assistant Professor of Histology and Embryology, *School of Medicine, Western Reserve University, Cleveland, O.*
- BARRIS, RALPH W., Ph.D.*
- BARRON, DONALD H., B.A., M.S., Ph.D., Associate Professor of Physiology, *Yale University School of Medicine, New Haven, Conn.*
- BARRY, ALEXANDER, A.B., M.A., Ph.D., Instructor in Anatomy, *University of Michigan Medical School, 1430 Golden Ave., Ann Arbor, Mich.**
- BARTELMER, GEORGE W., Ph.D. (Vice-Pres. '32-'34, Exec. Com. '36-'40). Professor of Anatomy, *The University of Chicago, Chicago 37, Ill.*
- BARTSCH, PAUL, B.S., M.S., Ph.D., D.Sc., Curator of Mollusks and Cenozoic Invertebrates, *U. S. National Museum, Washington, D. C.*
- BASSETT, DAVID L., A.B., M.D., Assistant Professor of Anatomy, *Stanford University, School of Medicine, Stanford University, Calif.*

- BAST, T. H., B.A., Ph.D., Professor of Anatomy, *University of Wisconsin, 308 Science Hall, Madison, Wis.*
- BATSON, O. V., A.M., M.D., Professor of Anatomy, *Department of Anatomy, Graduate School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.*
- BAUMGARTNER, WILLIAM J., A.B., A.M., Ph.D., Professor of Zoology, *University of Kansas, 1209 Ohio St., Lawrence, Kan.*
- BEAMS, HAROLD W., Ph.D., Professor of Zoology, *State University of Iowa, Iowa City, Ia.*
- BEATON, LINDSAY E., A.B., M.S., M.D., Medical Fellow, National Research Council, *Northwestern University Medical School, Chicago, Ill.**
- BEATTIE, JOHN, M.D., D.Sc., Conservator of Museum and Director of Research, Royal College of Surgeons; Colonel, Army Medical Service; *Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2, England.*
- BECKER, R. FREDERICK, B.S., M.S., Ph.D., Instructor in Microscopic Anatomy, *Northwestern University Medical School, Chicago, Ill.*
- BÉLANGER, LEONARD F., A.B., M.D., A.M., Assistant Professor of Histology and Embryology, *University of Montreal, Montreal, Canada.**
- BENJAMIN, JAMES W., A.B., M.S., Ph.D., Associate Professor of Histology, Embryology and Neuroanatomy, *New York Medical College, Flower and Fifth Avenue Hospitals, New York City.*
- BENNETT, GEORGE ALLEN, A.B., M.D., Associate Professor of Anatomy, *Daniel Baugh Institute of Anatomy, Jeerson Medical College, Philadelphia, Pa.*
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TERMS OF POSITION AND DIRECTION IN THE NK-INA REVISION OF THE BASLE NOMINA ANATOMICA

It will be recalled that a Commission on Anatomical Nomenclature was appointed by the International Congress of Anatomists, at Milan, in 1936, to undertake the task of bringing some order out of the confusion which had been created by the introduction of British and German revisions of the Basle Nomina Anatomica. Those revisions were sponsored by the Anatomical Society of Great Britain and Ireland and by the Anatomische Gesellschaft and are, respectively, the British Revision (BR) and that of the Nomenklatur Kommission (NK). The subsequent revision of the latter is known as the Jena Nomina Anatomica (JNA or INA).

The Commission accepted the NK-INA list of anatomical names as the basis for the official International Revision which it proposed to draw up and submit for the consideration of, and possible adoption by, the International Congress. The work of the International Commission was interrupted by the war; but it is hoped that it will be resumed as soon as conditions permit. It had been originally intended that its recommendations be considered at an International Congress in London, in 1939.

In our previous communications, we stated that we are opposed to the use, in textbooks and elsewhere, of anatomical terms which are not included in the BNA, before such terms have been approved by authorized representatives of the various national anatomical societies. The introduction of new anatomical terms which might subsequently be superseded by others adopted by the International Congress is, it seems to us, a highly inadvisable procedure, and one which is almost certain to increase the synonymy and confusion which the original BNA did so much to reduce.

We agree, however, with those who have attempted revisions of the BNA, in believing that some changes in the original nomenclature ought to be made. We believe further, that before the next International Congress of Anatomists, at which, presumably, the changes in nomenclature recommended by the Commission will be considered, it would be well if the members of the American Association of Anatomists who cared to do so had an opportunity to state their views on the desir-

ability of the changes which are contained in the proposed revisions of the BNA. The purpose of this communication is to ask for an expression of opinion from anatomists in this country concerning that portion of the BNA, and of its revisions, which deals with terms of position and direction. We should welcome, too, their reactions to the views expressed in the following paragraphs.

Should certain terms of position in human gross anatomy continue to have their basis in that rather artificial concept, the "anatomical position," in which the subject is described as standing erect, with arms hanging at his sides, and the head, eyes, and palms directed forward? The cadaver is never dissected when in that position; the physician and the surgeon deal mostly with patients who are supine; and, if either of them were confronted in his office by a man who approached him with his palms directed forward, he would probably leave hurriedly by the nearest exit.

Von Koelliker and Krause who were, respectively, chairman and editor of the original BNA commission, urged the adoption of terms of direction and position which would be independent of the posture of the body; but the majority of the members did not concur with their view. His, the author of the extensive report which accompanied the BNA list when it was submitted to the Anatomische Gesellschaft, agreed that some advantages would result from the adoption of Krause's and von Koelliker's suggestions, but held that such an action would be "precarious," in that it would involve too radical a departure from traditional usage. He commented further, that "it is for time to decide whether or not it will definitely break from the conventional custom of referring to the upright position of man."

It appears that that decision has been reached by the authors of the NK-INA nomenclature. That terminology retains *medialis* and *lateralis* of the BNA, but substitutes *ventralis* and *dorsalis* for anterior and posterior, and *cranialis* and *caudalis* for superior and inferior, as terms of position and direction to be used in the description of the trunk and neck. Similarly, *ventralis* (or *volaris*) and *dorsalis*, *radialis* and *ulnaris*, *fibularis* and *tibialis*, and *proximalis* and *distalis* are substituted for anterior and posterior, *lateralis* and *medialis*, and superior and inferior, as terms to be applied to the extremities. *Frontalis* and *occipitalis*, *temporalis* and *nasalis*, and *maxillaris* and *mandibularis* are recommended as the corresponding terms for the head.

If these suggested changes are adopted, they can either be used exclusively as terms of position and direction for the appropriate parts

of the body (head, neck and trunk, and extremities), or they can be employed also to designate branches of vessels and nerves and surfaces, margins, and other aspects of various other organs. The first alternative would leave us with such incongruities as anterior and posterior branches of vessels and nerves which course towards surfaces which have been officially designated ventral and dorsal. The other alternative is to rename all structures to conform to the revised terminology. The latter course has been followed to some extent in the NK-INA revisions; and the resulting changes should be considered in any appraisal of the desirability of the recommended terms of position and direction.

The Stanford group is not in unanimous agreement on this question. One member of the staff favors the retention of the older terminology on the grounds that: (a) it has worked satisfactorily over a long period of years; (b) it is firmly entrenched in the literature and is familiar to most physicians and anatomists; and (c) any attempt to apply the new terminology consistently to other structures would result in much confusion. Another member of the group suggests that both sets of terms be officially approved so that one would be free to use whichever he preferred. The majority, however, would urge the adoption of the NK-INA terms of position and the renaming, in so far as practicable, of various anatomical structures so as to conform to the new terminology.

In reaching this decision we have been guided by the following considerations: (a) it is advantageous to use terms of position and direction in human gross anatomy which are literally accurate regardless of the posture of the body at the time they are applied; (b) to the extent to which such terms are the same as those employed in comparative vertebrate anatomy, their use would serve to emphasize the homologies between human and other vertebrate forms; and (c) the fact that a group of terms have been employed for more than half a century is not in itself enough to justify their perpetuation, especially when (as we believe) the advantages which would result from the suggested changes in terminology outweigh the temporary inconveniences which their adoption would entail.

This statement of the position of the majority of the Stanford group on the desirability of suggested changes in terms of position and direction is presented here in the hope that it will serve to stimulate discussion of the proposed revisions of the BNA, in the pages of this journal. Though the view expressed is based on our considered judg-

ment, we should not urge it, if it proves unacceptable to the majority of anatomists. In this, as in other questions of nomenclature, we should gladly defer to the preference of the majority of our colleagues in anatomy throughout the world, to the end that a satisfactory and universally acceptable revision of the BNA may be achieved.

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THE MIDBODIES IN HUMAN ERYTHROBLASTS

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NINETEEN FIGURES

The remarkable structure known as the "midbody" appears in the latter part of the anaphase of mitotic division. It develops from thickenings in the exact middle of the "connecting" or "interzonal" achromatic fibers. These thickenings are transformed into discrete deeply stained granules, situated in the equatorial plane (the future cytoplasmic division plane) and form a configuration very similar to the cell plate of plant cells. As the chromosomal asters separate, the bundle elongates. With the approach of the divisional furrow it becomes constricted so that in the end the granules are compressed and coalesce into a few granules or a single coarse granule lying in the narrow bridge formed by the residues of the interzonal fibers and uniting the daughter cells. The term of "midbody" (Zwischenkoerper) was coined by Flemming for the granules in that last position, whereas the origin from an equatorial structure was described later. The term thus does not quite cover the developmental configuration. That is the reason for some more differences in the use of the term, which was extended to the whole sequence of the granule formation or even comprises the interzonal bundle (Wassermann, '29) or restricted to its original significance. The equatorial arrangement of the granules was referred to as "cell plate" (Wilson, '25) or "plaque fusoriale or cellulaire" (Carnoy) or "central spindle bodies" (Zentralspindel-Körperchen, Kostanecki) or halving nodules (Halbierungs Körperchen, Ballowitz). These extensions and specifications reflect the growing acknowledgment, that we face in this structure as complicated mechanism linked with mitotic division.

Besides the numerous studies, directed especially to the midbodies, the latter are frequently represented in illustrations without further comment. Fry ('37) has collected many such instances. The midbodies were demonstrated in the maturation divisions of ova and spermatocytes and in the cleavage of eggs in Mollusca, Echinodermata, Dicyemides, Nemertines, Annelides, Arthropodes and Prechordates (in epithelial cells of Salpa). In vertebrates they were seen in brain cells of

fish-embryos (*Squalus*), in epithelial cells and in nonstriated muscle cells of *Amphibia* larvae (*Salamandra*, *Triton*, *Amblystoma*), in erythroblasts of the duck embryo and in amnion cells of the rat. In spite of this wide spread occurrence, the universality of the configuration and thereby its integral role in mitotic division is not yet unanimously accepted, so that any further contribution, especially referring to more specialized cells of higher animals seems to be desirable. Therefore the occurrence of midbodies in human erythroblasts may be worth description.

While investigating the gradual loss of the divisional capacity during the maturation of the human erythroblast, attention was directed to the behavior of the centrosomes and centrioles, for which purpose the Heidenhain iron-hematoxylin stain was used. Some of the specimens examined presented so many and excellent examples of midbodies, that the process of their formation and their fate could be followed exactly.

The specimens in question were material obtained by sternal puncture from two cases of untreated pernicious anemia, the one fixed in Zenker-Formol, the other in concentrated mercury bichloride, both fluids containing 5% of glacial acetic acid. The paraffin sections of 5 microns were stained by the Heidenhain iron-alum-hematoxylin method, the differentiation was done with a 2% solution of the iron-ammonium-sulfate. The marrow of pernicious anemia is highly hyperplastic, the erythrocytic tissue in the state of maximal proliferation, although the erythroblasts do not belong to the normoblastic type but are megaloblasts, a pathological modification of the first and specific for pernicious anemia. The abnormal size of these cells, by far surpassing that of the normoblasts, probably accounts for the striking visibility of the midbody formations. The cytological identification of the cells in mitosis, especially the differentiation from dividing myeloblasts in such preparations is very difficult and can only be done by the simultaneous study of neighboring sections stained with a Romanowsky stain and besides requires a thorough familiarity with the matter. The abnormal size of the dividing megaloblasts compared with that of myeloblasts facilitates the identification but this help does not hold throughout, because hypertrophic cells of the myeloid branch are common in the bone marrow of pernicious anemia. These obstacles, however, do not alter the value of the observations, which consists in the demonstration of midbodies in specialized human cells.

Although not all cells in late anaphase or telophase show midbodies it does not follow, that the latter represent an accidental feature of mitotic division. Uncontrollable local differences in the action of the fixation are responsible for such defects, as was remarked by Fry ('37). Furthermore, the differentiation by the iron-alum solution, as careful we may proceed, is never homogenous on a slide. Slight differences in the thickness of the sections and local irregularities in one section are the causal circumstances. The insignificance of such failures may be inferred from the fact that some cells may show distinct spin-

dles or interzonal bundles, whereas cells in identical phase, lying in the immediate vicinity show no trace of these indispensable constituents. The same may be said of centrioles and polar bodies, the staining of which is as delicate and irregular as that of the midbodies.

OBSERVATIONS

1. The first trace of the midbody appears in later anaphase, when the two asters have moved further apart and the interzonal fibers became clearly visible. In that stage the peripheral fibers of the bundle are curved with the convexity to outwards, so that the bundle assumes a barrel-like outline. Towards the center of the bundle the curvature diminishes gradually, so that the innermost fibers run straight from

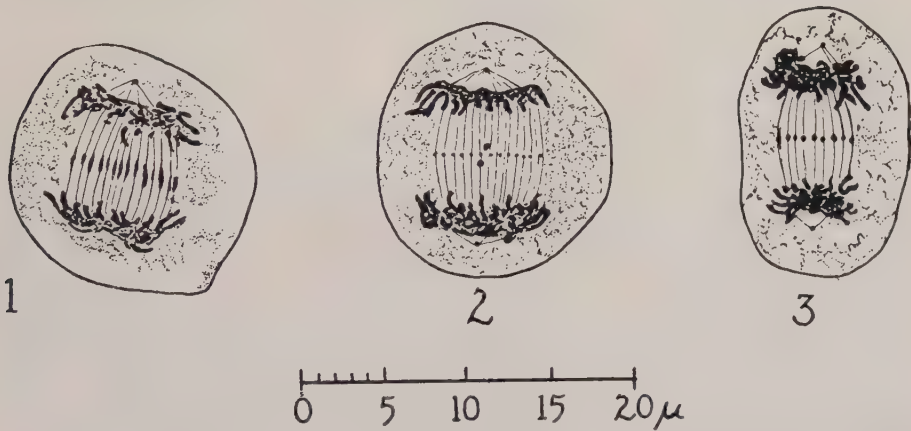


Fig. 1 Initial thickening of the interzonal fibers in the equatorial plane, the future division plane.

Figs. 2 and 3 First appearance of the midbody granules on the site of the thickenings. In the figures 1-3 the interzonal bundle is barrel shaped.

aster to aster. The interzonal fibers are coarser than those of the polar spindle. Exactly in their halving point the fibers show a slight fusiform thickening, which retains a faint hematoxylin stain. The thickenings are arranged in a horizontal line, perpendicular to the bundle axis, indicating the situation of the former equatorial (i.e., the future divisional) plane, although no trace of the constriction of the cell body is yet recognizable (fig. 1). In a slightly later stage the interzonal bundle has begun to elongate but preserves the barrel-like outline though the curvature is more flat than before. At the site of the thickenings deeply stained small granules appear, forming a straight line across the middle of the bundle. The cytoplasmic constriction has not yet set in (figs.

2, 3). It can be definitely made out, that every granule lies in the middle of an interzonal fiber, although every fiber may not have a granule. By altering the focal distance there appear in some instances one or more indistinct granules on a lower or higher level, but the object is so minute, that the slightest movements of the micrometer screw are too gross for a distinct localization of these granules in corresponding fibers. The number of sharply defined granules in the equatorial plane varies between five and ten and we must consider the possibility that in the sectioning a layer of the bundle may be cut off, so that some of the granules lying in other levels are withdrawn from observation.



Fig. 4 Stretching of the bundle. Beginning indentation of cytoplasm. Numerous midbody granules.

Fig. 5 Elongation of the bundle; deeper cytoplasmic indentation.

Fig. 6 Progressive indentation, forming a deep furrow on one side.

2. With the beginning elongation accompanying the moving apart of the asters, the interzonal bundle becomes more and more cylindric by the stretching and flattening of the curved peripheral fibers (figs. 4, 5 and 6). The divisional furrow, at first recognizable as a superficial indentation (figs. 4, 5) deepens more and more until it touches the interzonal bundle (figs. 6, 7). In some instances, the indentation appears shallow in spite of a distinct line apparently separating the cells. This line corresponds just to the perspective projection of the shallow furrow running around the cell and indicates a condensation of the cytoplasm in that region (fig. 8).

3. When the furrow reaches the bundle, the latter begins to constrict exactly in the division plane and eventually assumes the shape of a double truncated cone (figs. 10, 11 and 12). With progressing elongation the bundle becomes more ribbon-like and its equatorial indentation flattens, thus forming the "connecting stalk" (figs. 11, 12) of

Chambers. Finally the bundle is reduced to the connecting bridge between the daughter cells (figs. 14, 15) which disappears in the end. At that phase the intracellular residues of the stalk have also disappeared.

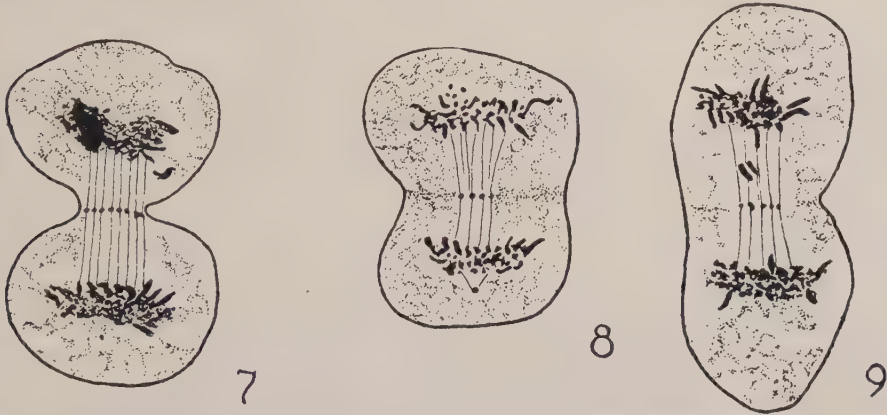


Fig. 7 Elongated bundle. The divisional furrow reaches the bundle. Numerous midbody granules.

Fig. 8 Beginning constriction of the bundle. Condensation of the cytoplasm in the plane of the furrow. Fewer but coarser midbody granules.

Fig. 9 Elongated bundle (connecting stalk). Two aberrant chromosomes.

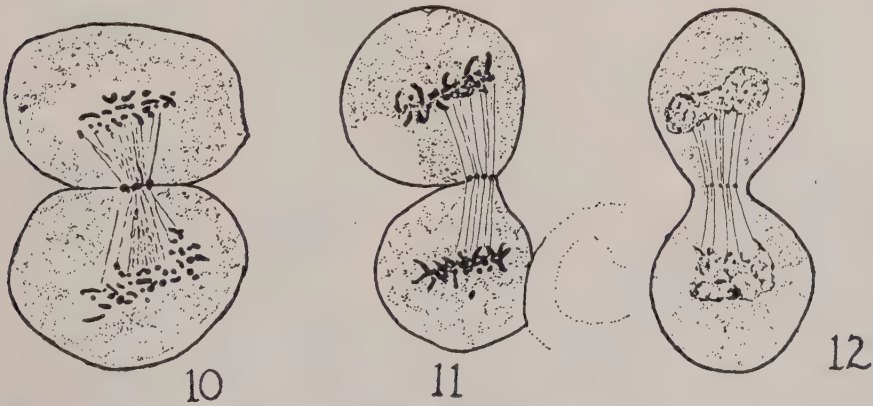


Fig. 10 Deep constriction of the connecting stalk. Few and coarse midbody granules.

Fig. 11 Long connecting stalk. Few midbody granules in the uniting bridge.

Fig. 12 Late telophase. Nuclear membrane in formation. Connecting stalk with few midbody granules in the furrow plane.

A strict correspondence between the constriction of the interzonal bundle and the nuclear reconstruction cannot be established. The process begins in the late anaphase and goes on till beyond the dispireme of telophase, sometimes reaching its end already in the beginning of the dispireme (fig. 15), sometimes leaving distinct fiber residues although the nuclei have already passed that stage (figs. 12, 13 and 14).

The midbody granules become crowded together during the constriction of the bundle (figs. 8, 9 and 10). Usually their number decreases to at most four to five (figs. 11 to 15) whereas they appear coarser than before, suggesting a fusion of the smaller primary granules. Yet in

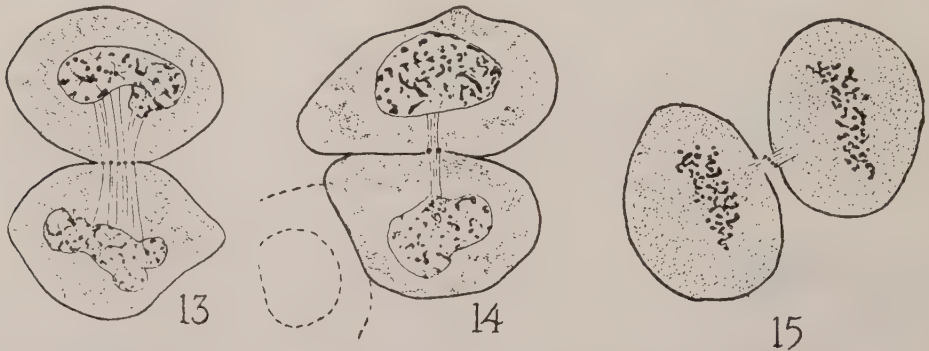


Fig. 13 Nuclei in reconstruction. The midbody granules in the uniting bridge.

Fig. 14 Same phase as in figure 13. The bundle contains only two fibers and two granules.

Fig. 15 Nuclei in dispireme. The uniting bridge formed by the residues of the bundle fibers. The midbody granules outside of the cells.

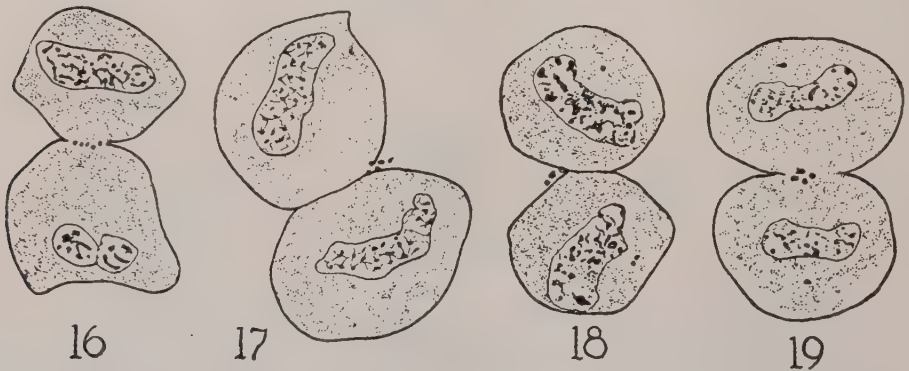


Fig. 16 Nuclei in reconstruction. The interzonal fibers no longer visible. The midbody granules in the furrow (perspective view).

Fig. 17 Same phase as in figure 16. The midbody granules in the furrow (side view).

Fig. 18 Same phase. Side view. Centrioles visible.

Fig. 19 Same phase. Perspective view. Centrioles visible.

cases like figure 14, showing only two granules and two fibers, it is possible that we are dealing just with a slice of the whole structure.

4. With the separation of the cells and the reduction of the bundle to a thin bridge the granules are situated in that bridge (figs. 14, 15 and 16), giving the picture of the midbody described as such by Flemming (1891) in his communication on the subject.

5. After the bridge and the intracellular residues of the interzonal fibers have disappeared, the midbody granules lie in the divisional fur-

row between the cells and outside of the cell body (figs. 17, 18 and 19). A picture like figure 19, where the granules seem to remain within the cell body is just a projective view.

DISCUSSION

It is beyond the purpose of this paper to enter in the physico-chemical theory and the significance of the midbody, a task requiring extensive studies on a large material. Only a few remarks concerning this side of the subject may be added as far as they can be based on the findings described above.

(a) The structures in question, as observed in human megaloblasts, harmonize in every detail with the descriptions and illustrations of the midbodies as previously observed in other animal cells. This constancy in such diverse material supports the assumption that we have in the midbodies a process of universal occurrence forming an integral part of mitotic division.

(b) The appearance of the midbody granules before the first signs of cellular constriction and the later onset of the divisional furrow in that level suggest, that the situation of the plane of division, identical with the former equatorial plane, is determined long beforehand. From the work of Nemeč ('28), Belař ('27, '29), Hartog ('04), Schrader ('44) a.o., and in order to account for the arrangement of the midbody granules in one plane, the assumption becomes probable that the whole process of mitotic division is governed by two spheric fields of forces, centered in the poles and neutralizing each other in the plane of their intersection.

(c) The final exclusion of the midbody granules from the daughter cells, well known in these observations, opposes the assumption of Benda and of Kostanecki that the midbody is in a way linked with the formation of the centrioles. This is clearly shown in the figures 18 and 19, where the extracellular midbody granules and the intracellular centrioles are simultaneously represented.

(d) Ballowitz (1898) thought that the initial thickenings in the halving points of the interzonal fibers, their concentration into the granules and the final expulsion of the latter express a metabolic process, eliminating some material no more utilized by the cells. The pictures as formed in the megaloblasts agree with this conception. Moreover, they support the homology of the initial configuration of the midbody in animal cells with the cell plate of plant cells. The difference consists in the fact, that in the latter the structure is used in building up the cell wall, whereas in animal cells having no cell membrane it is eliminated as a waste product. Nevertheless, we are not justified conceiving the

process in animal cells as a mere phylogenetic rudiment. It is more probable that the process belongs to the essential mechanics of mitotic division and that in animal cells only the last link in the chain of phenomena has been altered in the sense of a functional transformation. We have no reasons to assume that the expulsion of the substances carried by the midbodies is a meaningless residue.

(e) Fry ('37) has advanced the theory, that the midbodies represent but an accumulation of dye substance in "points of focalization", i.e., in places where fibers either cross each other or are pressed together by a constricting mechanism. Schrader ('44) opposed this explanation pointing to instances, mentioned by Fry himself, where no constriction is at work. The observations described above show clearly that the "cell plate", formed by the midbody granules is distinctly present long before any sign of constriction of the interzonal bundle, as demonstrated by the barrel shape of the bundle. Since the terminal midbody granules lying in the achromatic bridge or after its disappearance outside of the cells are doubtless the continuation of the granules in the cell plate the explanation of Fry is not applicable to that object here.

SUMMARY

The presence of typical midbodies in human erythroblasts (megalo-blasts) is demonstrated and their development followed in detail.

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THE EMBRYOLOGICAL DEVELOPMENT OF THE WOLFFIAN AND MÜLLERIAN DUCTS AND THE ACCESSORY REPRODUCTIVE ORGANS OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*)¹

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ONE PLATE (SEVEN FIGURES)

INTRODUCTION

The golden hamster (*Cricetus auratus*) is being widely used in sex research. As has been pointed out by Bond ('45) in her report on care, breeding, and growth of this animal, it offers advantages as a laboratory mammal. It is easy to breed in captivity, is very prolific, and has a short gestation period of less than 16 days.

In spite of its wide use, its embryology has not been thoroughly described. Graves ('43) has studied the early embryology covering the first 9 days post coitum. In his preliminary report, he comes to the conclusion that the hamster develops faster than any other placental mammal, and just as fast as the opossum. In view of this rapidity of development, the embryology of the reproductive system, and especially that of the wolffian and müllerian ducts and the accessory reproductive organs, is of particular interest. Therefore, such a study has been undertaken, mainly as an introduction to the problem of the later development of the reproductive system after birth, and the sensitivity of this system to hormones. Research on the postnatal development and sensitivity of the gonads and accessory organs is already in progress.

MATERIAL AND METHODS

This research is based on data from thirty-two animals, of which twenty-five were embryos and seven were between birth and 6 days of age. The age of the embryos was computed in days and hours after observed copulations. The youngest embryos were 10 days post coitum.

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Four specimens were studied at this age; three at 10 days and 12 hours; eight at 11 days and 19 hours; two each (one male and one female) at the ages of 12 days and 21 hours, 13 days and 20 hours, 14 days and 12 hours, 14 days and 21 hours, 15 days and 12 hours, at birth, and at 5 hours after birth. Two females and one male were studied at 6 days of age. Animals of the same age were littermates with the exception of a few cases.

Embryos were removed from the mother, freed from their membranes, and fixed in Bouin's fluid. The whole embryo was sectioned in the younger ages, but only the posterior half or the urogenital region in older specimens. All the material was cut serially at a thickness of 10μ and stained in Harris haematoxylin. The length of the wolffian and müllerian ducts, as well as that of the accessory reproductive organs, was computed by counting the sections in which these structures appeared and multiplying by the thickness of the sections.

The main emphasis of this study was placed on the development of the wolffian and müllerian ducts, and the accessory reproductive organs—prostate, seminal vesicles, coagulating glands, bulbo-urethral glands. The gonads were observed only superficially, from the point of view of sex differentiation.

OBSERVATIONS

Ten days post coitum. A study of the hamster embryo at 10 days post coitum reveals the reproductive system in a very primitive condition. It consists of an accumulation of epithelial cells forming a very small and indistinct genital ridge, which is beginning to separate by a shallow groove from the mesonephric fold. There is no müllerian duct present at this age. The wolffian duct, however, being part of the primitive urinary system, is already well developed, and has a lumen. It runs posteriorly to the cloaca, which it joins, although the junction is solid.

The mesonephros is very small and is located ventral and medial to the post-cardinal vein in the urogenital fold. It has very few tubules, most of which are still solid.

The permanent urinary system is beginning to develop at this stage. The ureter is already branching off from the wolffian duct. It is a small hollow outpocket $150\text{--}170\mu$ long, running dorsal and cranial from its point of origin in the mesonephric duct. There is some condensation of tissue around the ureter which might be interpreted as the metanephric blastema.

Ten days and 12 hours post coitum. At the second age in this series, the gonad primordium is a little more advanced in its development. The genital ridge is more distinct, its division from the mesonephric fold being marked by a deeper groove. There is also a mass of cells forming a dark staining epithelial nucleus in the region where the sex cords will later develop. The müllerian duct is still completely absent. The wolffian duct has grown longer during this 12-hour period; however, its stage of development is not more advanced than at the earlier stage, and it is still solid where it joins the urogenital sinus.

The ureter seems to be growing very fast during these early stages. In 12 hours it has grown more than three times in length in some cases. Its lumen is also larger.

The cloaca is now almost completely divided into rectum and urogenital sinus.

Eleven days and 19 hours post coitum. A great advance in the development of the urogenital system has taken place since the last stage. The gonad has not only grown tremendously in size, but it is also beginning to differentiate sexually. Sex can be determined microscopically. As figures 1 and 3 show, the cells in the outside layers of the gonad in some embryos are beginning to elongate and assume the shape of tunica cells. There is also some tendency of the epithelial mass to organize into small cell clusters, thus suggesting the very beginnings of testis cords. On the basis of these findings, three of eight embryos were considered males, and the others females or undifferentiated as to sex. The indifferent gonad or ovary (fig. 2) consists of an epithelial mass surrounded by the germinal epithelium, but it has no tunica.

It is interesting to note that in these differentiating males the solid efferent ducts of the mesonephros are already sending downgrowths into the gonad, which connect with the solid rete, and so form the urogenital connection. In the female or undifferentiated gonad, however, this connection is not very clear in most cases.

Though the gonads at this age are beginning to differentiate, the ducts do not show any difference in the two sexes. As can be seen in figures 1 and 2, the wolffian duct is as well developed in the sexually undifferentiated embryo as it is in the differentiating male. It has grown about as much in one as in the other. It is patent throughout, but still joins the urogenital sinus without a lumen at the junction. The müllerian duct is now present for the first time. It is shorter and narrower than the wolffian duct. Its anterior end has an ostium about 50 μ in length on the ventral side of the mesonephric fold. The duct follows

antero-posteriorly as a narrow solid cord in some regions, though in others it is beginning to acquire a small lumen. It is located at the outer tip of the mesonephric fold. At this age it has not developed any farther posterior than the region of the gonads. There is no visible difference between the müllerian duct of the differentiating male and that of the undifferentiated or female embryo (see figs. 1, 2).

The wolffian body has also increased in size since the last age. It has more tubules and they all have lumina. Most of these tubules join the wolffian duct.

The metanephros has developed greatly in the last 30 hours. It is a fairly large body in the region of the mesonephric fold, and lies medial to it. It has many hollow branching tubules. The ureter is very long and is still attached to the wolffian duct just before the latter joins the urogenital sinus. This junction is still solid.

Twelve days and 21 hours post coitum. Sexual differentiation has advanced rapidly. Sexes can be detected very readily by microscopic examination of the gonad. The tunica albuginea of the testis is now a very distinct band, several layers thick, of lighter staining elongated cells between the germinal epithelium and the epithelial mass. The cell clusters in the epithelial mass are beginning to round up gradually into testis cords, but these are not definitely distinguishable as such until 24 hours later. The epithelial mass of the ovary is also rounding up into cell clusters, which are separated from each other by connective tissue strands. The connection between efferent tubules and rete cords is still more marked in the testis than in the ovary.

The wolffian duct of the female is growing and developing as fast as in the male. It is larger in diameter than in the previous age, and has a large lumen. In both sexes it joins posteriorly the solid sulcus prostaticus.

As already mentioned in the previous age, the müllerian duct grows antero-posteriorly. This caudal growth has now progressed greatly, so that at this age the duct has reached the posterior end of the embryo and has the same length as the wolffian duct. Posterior to the gonads, the two mesonephric folds have now grown together in both sexes, and continue posteriorly along the midplane as the genital cord. As a result, the müllerian ducts come to lie side by side in the center of the cord, while the wolffian ducts appear lateral to them. The müllerian duct is exactly the same length in both sexes; however, it is beginning to show differences in the two sexes. In the male, this duct is already degenerating. This is detectable by the condition of its lumen, which

is present in only one-third of its length, while in the female a lumen is present throughout.

Posteriorly, the müllerian duct in both sexes seems to join the wolffian duct on each side before the latter joins the urogenital sinus. If this is the case, the wolffian duct widens tremendously and becomes solid for some distance anterior to the urogenital sinus. However, it is more likely that these wide solid structures joined by the müllerian ducts are not the widened wolffian ducts, but two solid dorsal projections of the urogenital sinus, known as the prostatic sulci, which the wolffian duct has already joined.

The bladder is now formed and the ureters join it, but this junction is still solid.

Thirteen days and 20 hours post coitum. The testis cords, which have been gradually developing, are now definitely formed. There is a marked contrast between the tissue forming the cords and the interstitial tissue between them. The ovary does not show any further development, except for a more marked cluster arrangement of cells in the epithelial mass. The rete and the efferent ducts of the testis are developing very slowly and show no conspicuous further differentiation at this age. In the female, however, the rete seems more developed and its connection with mesonephric tubules more marked than at earlier stages.

The wolffian duct is now beginning to show sex differences. It is longer and more developed in the male. In the female it is degenerating; is absent at intervals along its course; and is much smaller in size than the müllerian duct.

The müllerian duct, which had started degenerating in the male 24 hours earlier, shows further degeneration. Its anterior end is disappearing and its lumen is now obliterated except in the region of the genital cord. In this region, the right and left ducts come to lie side by side, as explained above. In the female the müllerian duct is very well developed. In both sexes this duct still seems to join the wolffian duct on each side before reaching the urogenital sinus. However, the same explanation holds here as in the previous age, that the solid structure it joins may not be the expanded end of the wolffian duct, but the solid sulcus prostaticus of the urogenital sinus.

At this age the primordia of the accessory reproductive structures are beginning to develop. The first ones to appear are those of the bulbo-urethral glands. In both sexes they emerge from the posterior region of the urethra as two small solid buds ($40\ \mu$ in length).

Fourteen days and 12 hours post coitum. The testis has now descended to the region of the bladder. The ovary has also descended and is now just posterior to the kidney. The testis cords, as well as the sex cords of the ovary, have advanced very little since the last age. In the male, the wolffian duct is very well developed, while in the female, degeneration has proceeded roughly antero-posteriorly at great speed, so that only three pieces remain. The first is a solid remnant of the right duct about $170\ \mu$ long directly posterior to the gonads. The others are remnants of both right and left ducts ($230\ \mu$ long) in the extreme posterior end. They have lumina and still join the prostatic sulci.

The müllerian duct in the male is also degenerating antero-posteriorly; as was shown in the preceding age. In this specimen, however, a solid remnant of $200\ \mu$ appears in the region of the gonad on each side. In the genital cord patent sections of $580\ \mu$ are present. In the female, the utero-vaginal canal has not formed, although the right and left ducts are in close contact. In both sexes the müllerian duct joins the solid sulcus prostaticus medially and posterior to the junction of the wolffian duct.

The buds of the Cowper's (or male bulbo-urethral) glands at $14\frac{1}{2}$ days are still solid, and they appear to be six times as long as the ones at the preceding age. There is a condensation of tissue around each bud of the gland, the significance of which is not clear. In the female, the Bartholin's glands, the homologues of the Cowper's glands, degenerate almost immediately. In the previous age they were exactly the same size as those of the male; 16 hours later their presence is very doubtful, and thereafter, they are definitely absent.

Another interesting point in embryos at this age is the appearance for the first time of the anlage of the seminal vesicles in the male, with no homologue in the female. These glands are outpockets of the wolffian duct in the region of the genital cord. They grow in a dorsal and cranial direction and are lined with the same tall columnar epithelium of the wolffian ducts. The wolffian duct in the male is very well developed at the site of formation of the seminal vesicles. In the female, however, it is degenerating antero-posteriorly and is already absent in the region where the seminal vesicles would develop. Therefore, no homologues of such glands appear.

The mesonephros at this age is disappearing and only very few inconspicuous tubules are left. The ureters have lumina at their junction with the bladder.

Fourteen days and 21 hours post coitum. The sex cords and the rete in the testis and ovary do not show any further conspicuous development. Moreover, the degeneration of the ducts in opposite sexes has not gone any farther at this age than at the previous age.

There is some further development, however, in the accessory reproductive glands. The anlage of the Cowper's gland has now a very distinct duct coming from the urethra. Their homologue in the female is absent. The first anlagen of the prostrate gland in the male appear at this age. One dorsal and one ventral prostate bud 70μ long emerge from the margin of each sulcus prostaticus. As shown in figure 4, they are very small, round, solid buds. Their homologue is not present in the female at this age.

Fifteen days and 12 hours post coitum. The degeneration of the wolffian duct in the female has progressed farther posterior, so that only 70μ are left at the extreme posterior end. The müllerian duct in the male has not degenerated any further than in the previous age. It is more definite at this age that both ducts join the solid sulcus prostaticus separately, the junction of the wolffian duct being anterior to that of the müllerian duct.

The Cowper's gland has now a well developed duct which measures 130μ . The seminal vesicles have not grown in length but they seem to have a larger lumen. A pair of small solid buds can be recognized extending cranially from the ventral side of the sulcus prostaticus into the genital cord, at the point where the wolffian duct joins the sulcus. These seem to be the coagulating gland anlagen. The prostate gland has now a second pair of solid dorsal buds posterior to the first pair, and of the same length — 30μ . The ventral buds appear as in the previous age. In the female, however, there is still no indication of a prostate gland.

Birth (15 days and 16 hours post coitum). The embryos studied at birth had a rather short gestation period as compared to the mean found by Bond ('45) of 15 days and 21 hours. The condition of the testis and ovary does not indicate any further marked development of the cords. The rete is solid in both sexes, and in the female its connection with the efferent ducts is still not very conspicuous, though it seems to be present. The ovary is now completely surrounded by the ovarian capsule.

The ducts show no further degeneration in the opposite sex than in the previous age. In the male the remnants of the müllerian ducts form a uterus masculinus at intervals, then separate again into right and left tubes. In the female, the two müllerian ducts have not definitely

fused to form the utero-vaginal canal, but the partition between them is degenerating.

The accessory reproductive glands, however, show some further development. Around the solid anlage of the Cowper's gland there is an accumulation of tissue the significance of which is not understood. The seminal vesicles have grown to $400\ \mu$ in length, but they still appear as simple unbranched outpockets of the wolffian ducts. The coagulating gland anlagen have grown cranially and are beginning to acquire a small lumen. Now they appear to join the urogenital sinus dorsally and slightly anterior to the point where the wolffian and müllerian ducts join it. The prostate gland in the male has now two pairs of dorsal buds ($30\text{--}80\ \mu$), and two pairs of lateral buds. The first pair in each case is anterior to the second. There is only one pair of ventral buds, $30\ \mu$ long. They are all very small and solid. Prostate anlagen appear now for the first time in the female. There are three small, solid ventral buds emerging from the urethra. The first bud occurs only on the left; posterior to this is a pair.

Five hours after birth. The rete testis is now beginning to hollow. In the female, the rete is solid and its connection with the efferent ducts is no more obvious than in previous ages. The remnant of the wolffian duct in the female is very small in diameter and has no lumen for most of its length. It is short and discontinuous and is not present at the posterior end. In the male there is a piece of the uterus maculinus, which joins the urogenital sinus. As shown in figure 5, the utero-vaginal canal is now well formed in the female.

In the Cowper's glands the formation of a lumen has progressed from the duct into the anlage of the gland itself. The accumulation of tissue around the gland primordium is still very obvious. This thickening of tissue is also present in the female, but no anlage of the Bartholin's gland is present. There is also some accumulation of tissue around the seminal vesicles. These are still unbranched and have not grown significantly since the last stage. There is a very short ejaculatory duct ($80\ \mu$ long).

The primordium of the coagulating gland appears thicker and more conspicuous, but it is about the same length as before. It still does not have a lumen throughout, but its connection with the sinus is now hollow.

The prostate gland anlagen are still very small, solid, and inconspicuous. The ventral buds of the male have not grown any larger, but there is now a thickening of tissue around each ventral bud. Anterior

to these ventral buds there is only one pair of very small, solid lateral buds about $50\ \mu$ in length. These are located in the region where the seminal vesicles branch off from the wolffian duct. Posterior to the ventral buds are the dorsal buds. There is only one pair of these and though they are also very small, they are the largest ones at this age ($100\ \mu$ long). In the female there is only one pair of ventral prostate buds (fig. 5). They are about the same length, but they extend farther out from the sinus and seem to be larger in diameter than those of the male.

An interesting observation is that in the region of the ovary a remnant of the mesonephros is still present and extends for $400\ \mu$.

Six days after birth. Male. At 6 days after birth, the testis still has solid tubules. However, a very conspicuous change has taken place since the last age. The nuclei in the tubules are now gradually assuming a peripheral arrangement, thus leaving a clear center of cytoplasm. The rete testis is very large and has a large lumen. It connects with numerous efferent ducts, which in turn empty into a very coiled ductus epididymis, and this into a well developed vas deferens, with a wide lumen. Emerging as an outpocket from the vas is the ampullary gland. It grows cranial to its point of origin. The seminal vesicles have already emerged at a point posterior to this. Their growth within the first 6 days after birth is very marked. They have not only increased greatly in length, but also in complexity. They are slightly coiled anteriorly and their lumen is branching. The ejaculatory duct is very short — $80\ \mu$.

The coagulating gland is moving slightly dorsolateral in its growth, so that at this age it is already growing close to the seminal vesicles. It is now completely hollow. The Cowper's gland is large and convoluted, and has a wide lumen.

The prostate gland grows tremendously during the first 6 days. It is now a large gland around the urethra and is arranged into four lobes. As seen in figure 6, the two dorsolateral lobes are much larger than the two ventral lobes. The left dorsolateral lobe in this specimen appears posterior to the other three. The ducts in all the lobes run posteriorly to join the urethra. The dorsolateral lobes have multiple ducts, which join the urethra dorsally or laterally for some distance. The ventral lobes also have multiple ducts. The first three ducts in these lobes join the urethra laterally, instead of ventrally, with the exception of one on the right side. The rest of the ducts, posterior to these, all join ventrally. Posterior to the four lobes of the prostate, there is a series of very small buds, most of which are solid. They are arranged around

the urethra at different levels in a dorsal, lateral, and ventral position. Their size and stage of development can be compared with the condition of the first buds of the male prostate (fig. 4). Whether these small posterior buds are prostate buds or buds of the urethral glands is difficult to determine.

There is a remnant of the müllerian duct in the form of a uterus masculinus which is about $110\ \mu$ long and has rather a wide lumen.

Female. In the ovary at 6 days the cells of the sex cords are undergoing rapid mitosis. The rete ovarii is solid. Connecting with it is the epoöphoron, which has a lumen and is $80\ \mu$ long. There is also a very distinct paroöphoron with a lumen and a total length of $320\ \mu$. It appears anterior to the ovary, but since it does not connect with the rete, it cannot be considered epoöphoron. Its anterior position is probably due to the rotation of the ovary. These mesonephric tubules connect with each other in some cases, but they do not all open into a common duct, therefore no trace of the wolffian duct is recognizable.

At this age the vagina can be distinguished from the uterus by its wider lumen. At its anterior end it has developed two lateral pouches on its ventral side which connect with the vagina by two narrow ducts. These are the vaginal pouches, which characterize the hamster female. In the adult, they appear at the extreme posterior end of the vagina.

The prostate gland in the female also grows a great deal during the first 6 days after birth. The anlagen occur around the urethra in the form of dorsal, lateral, and ventral buds (fig. 7). These buds extend through a length of $1080\ \mu$ along the urethra, but they do not form lobes as in the male. There are over forty buds of different sizes ranging from 30 – $240\ \mu$ in length. The larger buds are hollowed through their whole length, others are solid buds with hollow ducts joining the urethra, and the smallest are completely solid. Some are straight, others coil. The ducts all run posteriorly to join the urethra. There is no trace of the Bartholin's gland.

RESUMÉ

From this account, the development of the reproductive system of the hamster can be reviewed as follows:

Male. The first indication of a genital ridge occurs at 10 days post coitum, and becomes more definite 12 hours later when it has acquired an epithelial nucleus. The gonad forms soon after this age, and by 11 days and 19 hours, some gonads are differentiating into testes as shown by the beginning of the tunica layer. Two days later, at 13 days and

20 hours, the testis cords are definitely present. These cords remain solid through embryonic life, and though they are still solid at 6 days of age, their nuclei are already arranged in the periphery of the cord. The rete testis is solid until after birth, but its connection with some of the mesonephric tubules is established very early at the time of the beginning of sex differentiation.

The mesonephric duct is complete and has a large lumen by 10 days post coitum. At this early age it has already formed the ureters, as its first pair of outpockets. The male wolffian duct shows no superiority over the female until 13 days and 20 hours. From this age until birth it appears more prominent in the male. At $14\frac{1}{2}$ days it gives off its second pair of outpockets — the seminal vesicles. At birth and shortly after, the male mesonephric duct begins to grow tremendously in length and has a very wide lumen. The epididymis is differentiated from the vas as a long coiled region of the duct by 6 days of age. At this age the third pair of outpockets has formed from the vas as the ampullary glands.

The müllerian duct is not formed until the time of the beginning of sex differentiation, at 11 days and 19 hours. Twenty-six hours later, at 12 days and 21 hours, it begins to show signs of degeneration in the male. This degeneration is antero-posterior and continues rather rapidly, though it shows a great deal of individual variation. While some embryos at 13 days and 20 hours show anterior degeneration of this duct, others at $14\frac{1}{2}$ days still retain a large solid piece in the region of the gonads. The uterus masculinus formed by the fusion of the right and left müllerian ducts is present at birth and 6 days after.

The first accessory reproductive glands to develop in the male are the Cowper's glands. The anlagen are present at 13 days and 20 hours. These glands show a very marked growth for the first 16 hours, but after this their growth in length is very slow until after birth. The second glands to develop in the male are the seminal vesicles at $14\frac{1}{2}$ days. At 14 days and 21 hours the first buds of the prostate gland are formed (fig. 4). Few new buds appear at the next ages. The greatest advance in the development of this gland takes place between birth and 6 days. At $15\frac{1}{2}$ days the coagulating gland anlage is formed in the male. This gland develops rather slowly until after birth.

Female. At the time of the beginning of sex differentiation, 11 days and 19 hours, the female is not yet easily recognizable from the undifferentiated stage, so that embryos which are not definitely males can be either females or undifferentiated. Not until 12 days and 21 hours can

the ovary be recognized as such. At this same age the sex cords in the ovary are beginning to appear. These cords develop very slowly so that there is hardly any visible difference at older ages. In the embryo studied at 12 days and 21 hours a rather distinct band of cells is seen between the germinal epithelium and the epithelial mass. This was interpreted as a primary cortex, but its complete absence at older ages makes this interpretation doubtful. At birth the ovary is already surrounded by the ovarian capsule.

The rete ovarii remains solid throughout development. Though solid, its connection with the mesonephric tubules is obvious. This connection is not clearly visible until 12 days and 21 hours, that is, about 26 hours later than in the male.

The wolffian duct in the female begins to degenerate at 13 days and 20 hours, 1 day after the ovary can definitely be recognized. This degeneration proceeds roughly antero-posteriorly. However, in some embryos this duct is degenerating at irregular intervals along its length, while in others the posterior end piece is missing when there are still some remnants anteriorly. In the specimen studied at birth there is a piece of the right and left duct 80 μ long at the extreme posterior end, still joining the urogenital sinus. Five hours after birth there is an anterior remnant of this duct on each side, and no posterior piece remains. Six days after birth, however, these remnants have completely disappeared, leaving no trace of this duct in the female.

The müllerian ducts do not fuse to form the utero-vaginal canal until after birth, though the partition between the ducts is very thin for about 20 hours before. At 5 hours after birth, the canal is definitely present for the first time (fig. 5). Six days after birth the vagina is distinguishable from the uterus and has the two lateral vaginal pouches.

The only male accessory glands which have homologues in the female are the Cowper's and the prostate. The Cowper's homologue, or Bartholin's gland in the female, develops first, and at the same age as in the male — 13 days and 20 hours. However, it is very short lived, since 16 hours later its presence is doubtful, and in 24 hours it is definitely absent. The prostate gland, however, persists for a longer time. It is first present at birth, which is approximately 20 hours after it appears in the male. Between birth and 6 days it undergoes great development.

DISCUSSION

The golden hamster has a very short gestation period compared to the 21- to 22-day-period in the rat and the 19-day-period in the mouse.

Bond ('45) has reported a mean period of gestation of 15 days and 21 hours, which confirms the findings of previous authors (Bruce and Hindle, '34; and others). The short gestation brings about a very rapid embryonic development. This has already been reported by Graves ('43) in relation to the early embryonic stages of this animal. From the preceding description of the development of its reproductive system, the rapidity of development is again very obvious. The very first indications of a genital ridge are present at 10 days post coitum, which means that the whole prenatal development of the reproductive system takes place in about 6 days. This is very much faster than in the rat or mouse. In the rat, the first indications of a genital ridge appear at 12 days and 18 hours (Price, unpublished), or about 9–10 days before birth. In the mouse (Brambell, '27 a) they appear at 9 days post coitum, that is, 10 days before birth. So, while the reproductive system of the rat has a prenatal development of 9 days and the mouse, of 10 days, that of the hamster is only 6 days. Such a discrepancy in length of time in the development of this system might lead us to expect a very primitive and undeveloped condition at birth, comparable to that of the opossum. However, such is decidedly not the case, since, except for the prostate gland, the genital system is, in general, no more primitive at birth in the hamster than in the rat.

Recognizable sex differences in the hamster appear less than 2 days after the first appearance of the genital ridge. In the rat (Price, '35, '36; Moore and Price, '42) visible male differentiation occurs 2 days after the genital ridge begins to develop, and in the mouse (Brambell, '27 a) sex differences are not detectable until $2\frac{1}{2}$ to 3 days after the ridge is present. Though development is a little faster in the hamster, the plan of sex differentiation corresponds closely to that of the other two animals. The first apparent differences in the sexes are seen in the gonads, the testis differentiating before the ovary. The ducts do not differentiate sexually until much later. It is interesting to observe that, as in the mouse (Brambell, '27b), the connection between the efferent tubules and the rete of the male gonad is established before that of the female. In fact, in the hamster this connection is present as soon as the testis begins to differentiate — 11 days and 19 hours.

The wolffian body and duct are completely established in the mouse before the reproductive system begins to develop, at 9 days post coitum (Brambell, '27 b). In the rat, they are already well developed by 11 days and 18 hours post coitum, that is, 24 hours before the beginning of the reproductive system (Price, '35). In the hamster, though the duct is

well developed by the time the genital ridge appears, the wolffian body has not developed very far yet but continues to grow and form more tubules for at least 2 days later. The degeneration of the wolffian duct in the female begins 2 days after sex differentiation is apparent in the gonad. This is exactly the case in the rat (Price, '35; Moore and Price, '42) and the mouse (Brambell, '27 b). Degeneration proceeds antero-posteriorly in the three rodents, though in both the rat and the hamster slight departures from this antero-posterior procedure are quite common. This degeneration occurs faster in the hamster. That is, the whole life span of the wolffian duct in the female rat after sex can be determined is about 6 days, since the last remnant of it is seen at 20½ days post coitum (Price, '35). In the hamster it lasts approximately 4 days. There is still a small piece left in the hamster 5 hours after birth, but its condition is so degenerate that it is doubtful that it would persist much longer. Other remnants of the wolffian system in the female hamster are the epoöphoron and the paroöphoron. They are still present at 6 days of age. In the rat, Price ('35) does not report any paroöphoron, but she found that the epoöphoron is present at 5 days of age. In the mouse, Brambell ('27b) found that the paroöphoron, degenerates early and cannot be detected at birth. The epoöphoron, however, persists throughout life.

The müllerian duct begins to develop in the hamster at the time that sexual differentiation is first apparent in the gonad. In the rat and the mouse, however, this duct is present somewhat in advance of the stage when the testis can be identified (Price, '35; Brambell, '27 b). In the hamster this duct grows very rapidly toward the posterior end, and attains its entire length in 1 day. Degeneration of the müllerian duct in the male starts very early. While the posterior end is still growing, the anterior portion is already losing its lumen, and so showing signs of degeneration. In the mouse (Brambell, '27 b) and the rat (Price, '35; Moore and Price, '42), degeneration of this duct in the male also begins anteriorly while the duct is still growing posteriorly. The condition of this duct in the male at birth and 6 days of age corresponds closely in the hamster and the rat.

Therefore, as can be seen from the above discussion of the development and degeneration of the wolffian and müllerian ducts in the rat, the mouse, and the hamster, the three animals agree remarkably closely.

In regard to the development of the accessory reproductive glands, the hamster also follows the pattern of the rat with some modifications. The order in which these glands begin to develop does not correspond

exactly. That is, in the hamster the Cowper's gland anlage appears first, and very soon after sex can be recognized. Both the male and female have the anlage of the gland, but, while in the male it grows and develops rapidly, in the female it disappears almost immediately. This is not so in the rat, since, according to Price ('35), it develops steadily in the female up to the first day of life. The second anlage to appear in the hamster is the seminal vesicle. This is the first in the rat, according to Price ('36). In all other details this gland develops similarly in both animals and reaches a comparable stage of development at birth and 6 days after. The fact that the seminal vesicle does not develop at all in the female rat is explained by Price ('36) by stating that this region of the wolffian duct in the female is already "missing or incomplete" before the time of development of these glands. This can be offered as an explanation also in the hamster, since the female wolffian duct is missing in the region where the seminal vesicles would be expected to develop.

The prostate gland is the next accessory gland to appear in the hamster. It has only about 1 day of prenatal life, during which its development is very slow. In the rat (Price, '36), it has about 2 days of prenatal life and has a very rapid development during this time. Consequently, the condition of the gland at birth in the male hamster is very much simpler and more undeveloped than in the rat. However, the condition at 6 days corresponds very closely with that of the rat at 5 days of age. A comparison of the prostate of a 6-day-old hamster (fig. 6) with that of the 5-day-old rat (fig. 5, Price, '36) shows very close similarity in degree of development. In regard to morphology of the gland, however, it is also obvious from these two figures that in the hamster the two ventral lobes are definitely separated from each other, but in the rat they are almost fused, forming one ventral lobe. This separation of the ventral lobes in the hamster persists throughout life.

The female prostate gland in the rat begins its development at the same age as the male (Mahoney, '40), or shortly after (Price, '36). It corresponds to only a portion of the ventro-lateral lobes of the male. The buds grow and coil slightly up to 5 days of age, according to Price. In the hamster, the female prostate does not appear until birth, that is, 1 day after it first appears in the male. Growth and development is also slight, but the condition at 6 days (fig. 7) shows a fairly well developed gland with both dorso-lateral and ventral buds. The buds are distributed through a long section of the urethra (1080 μ). Thus far, this gland has not been found in the adult female hamster. However, in the

rat well developed prostate glands are found in some adult females (Marx, '31, '32; Witschi, Mahoney, and Riley, '38; Price, '39).

Such a comparison of the hamster with the rat and the mouse brings us to the conclusion that, except for minor details, the development of the reproductive system of these three rodents corresponds remarkably closely. While the period of gestation of the hamster is about 6 days shorter than that of the rat, and 3 days shorter than that of the mouse, the speed of development is such that at the time of birth the stage of development of the reproductive system is essentially the same.

SUMMARY AND CONCLUSIONS

The embryology of the reproductive tract of the golden hamster was studied in thirty-two specimens, from 10 days post coitum to 6 days after birth. Emphasis was laid on the wolffian and müllerian ducts, and the accessory reproductive glands. The development of these structures was compared to that of the rat and mouse. The following conclusions are drawn:

1. The reproductive system develops faster in the hamster than in the rat and mouse.
2. The genital ridge is visible at 10 days post coitum.
3. The testis is recognizable at 11 days and 19 hours; the ovary 24 hours later.
4. The müllerian duct in the male begins to degenerate at 12 days and 21 hours, while the wolffian duct in the female starts degeneration 24 hours later.
5. The prostate gland begins to develop at 14 days and 21 hours in the male. It has a slow prenatal growth. In the female the prostate begins at birth. By 6 days it is quite well developed.
6. The seminal vesicles first appear at 14½ days; the coagulating glands 1 day later. These glands have no homologues in the female.
7. The bulbo-urethral glands begin to develop at 13 days and 20 hours in both sexes; they disappear soon in the female.
8. In general, the reproductive tract of the golden hamster at birth is as well developed as that of the rat.

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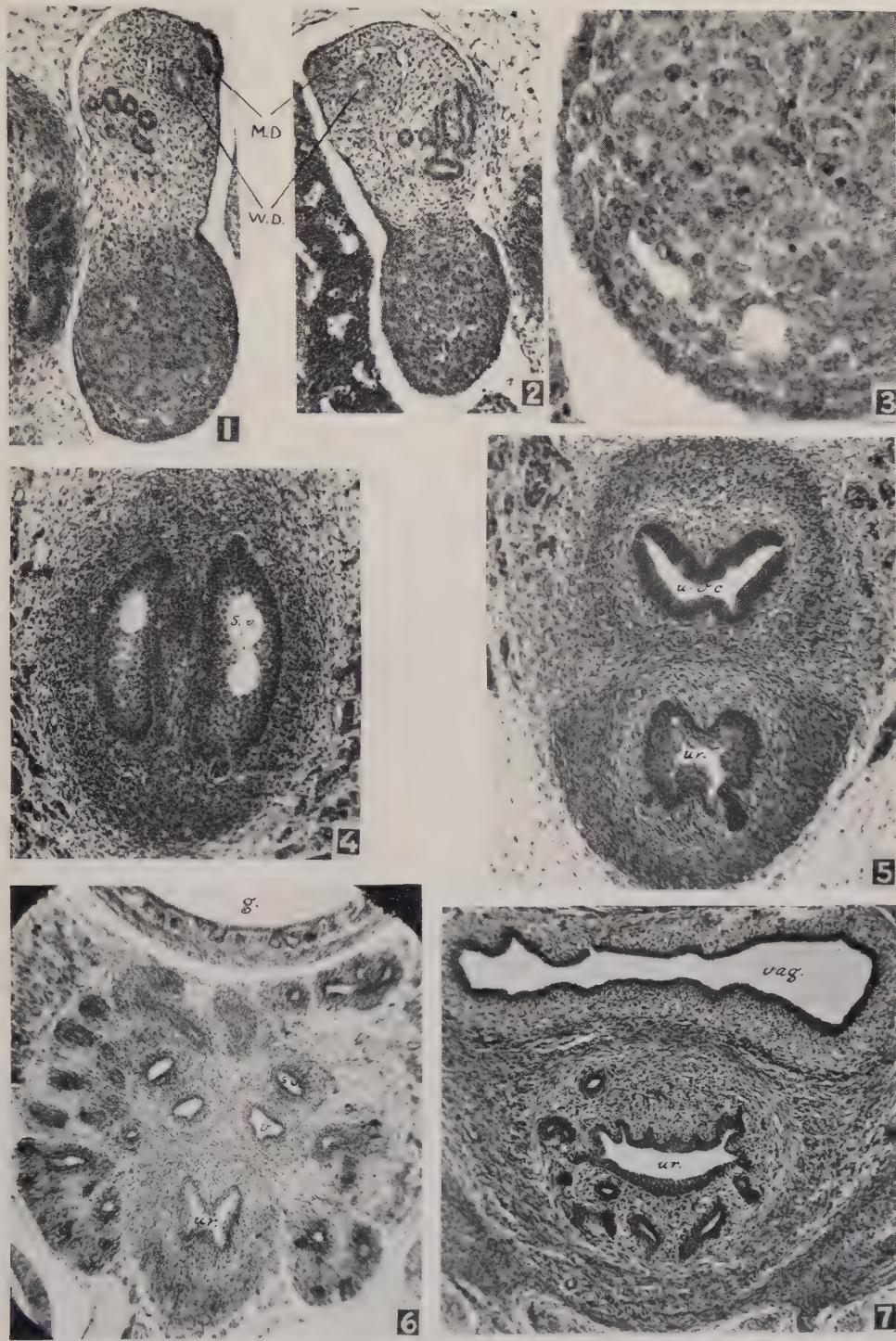
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PLATE 1

EXPLANATION OF FIGURES

- 1 Differentiating testis of a hamster embryo 11 days and 19 hours post coitum. MD, müllerian duct; WD, wolffian duct. $\times 86$.
- 2 Undifferentiated gonad, or ovary, of a hamster embryo 11 days and 19 hours post coitum. MD, müllerian duct; WD, wolffian duct. $\times 86$.
- 3 High power view of a portion of figure 1, to show developing tunica layer. $\times 309$.
- 4 Section through prostatic sulci of a male hamster embryo 14 days and 21 hours post coitum, to show the earliest buds of the prostate gland on the dorsal and ventral side of the sulcus. sp, prostatic sulcus. $\times 82$.
- 5 Section through the genital cord and urethra of a female hamster 5 hours after birth, showing the utero-vaginal canal (u-vc), and ventral prostate buds emerging from the urethra (ur). $\times 82$.
- 6 Section through the region of the prostate gland of a male hamster 6 days after birth. g, gut; sv, seminal vesicle; v, vas; ur, urethra. $\times 42$.
- 7 Section through the vagina (vag.) and urethra (ur.) of a female hamster 6 days after birth showing prostatic anlagen around the urethra. $\times 65$.



CHANGES IN THE RAT'S POSTERIOR PITUITARY FOLLOWING SODIUM CHLORIDE ADMINISTRATION

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FOUR TEXT FIGURES AND ONE PLATE (SIX FIGURES)

The intravenous infusion of hypertonic sodium chloride solutions into mammals causes a release of pituitrin (Gilman and Goodman, '37; Hare, Hare and Phillips, '43; Ingram, Ladd and Benbow, '39), and the response is sufficiently reliable to serve as an indicator of the animal's ability to produce pituitrin (Hickey and Hare, '44). The mechanism is activated by a rise in plasma osmotic pressure, possibly acting on the supraoptic nucleus (Chambers, Melville and Hare, '45). Correlation of this release of pituitrin with a cytologic change in the neurohypophysis has not been established. Gersh ('39) described intracellular osmiophilic droplets in the rat neurohypophysis which appeared to increase with water deprivation while the pressor, oxytocic and antidiuretic potencies of the lobe dropped. Gersh and Brooks ('41) found similar droplets in the posterior lobes of rats following stalk section and thought they represented the pituitrin left in the lobe after section. Hickey, Hare and Hare ('41) and Robertis and Primavesi ('42) also demonstrated these granules but could show no correlation between them and cellular secretory function. Selye ('43) injected 5% sodium chloride into rats and found generalized pituitary enlargement and a tense capsule with unusual prominence of the posterior lobe due to extensive stainable colloid secreted into the pituitary cleft. The gland from the salt treated rats was also hyperemic. Selye and Hall ('43) gave rats saline to drink, 1.5% for 2 weeks raised to 2.0% for 2 weeks and 2.5% for 2 more weeks. The posterior lobe was changed markedly.

“Even under low magnification this lobe appeared to be diffusely imbued with lightly staining edema fluid and in spots it exhibited a honey-combed appearance due to the presence of numerous vacuoles in the otherwise firmly packed posterior lobe tissue. Upon closer study, especially under high magnification, these small vacuoles were found to result from the presence of numerous pituicytes which were greatly enlarged and in the process of active mitotic proliferation. During mitosis the texture of the cell becomes extremely loose so that

the cytoplasm tends to shrink away from its surroundings and often cells are washed in the course of staining. In a good many cases, however, the cells engaged in mitosis remained attached to the slides and could readily be identified." (p. 580).

We had examined the pituitaries of salt treated or dehydrated animals (cats, dogs, rats) and had seen reticulated or foamy areas in the pars nervosa, but never attributed the vacuoles to the loss of mitotic figures in the preparation of the section. Furthermore, if these vacuoles are in reality lost mitoses, the rate of cell division in the posterior lobe of animals on a high salt intake must be far greater than ever observed in embryonic or malignant tissue.

The experiments of Selye ('43) and of Hall and Selye ('43) were repeated, and especial care taken in the histological preparation of the pituitaries to prevent the loss of cell parts by washing away from the surface of the slide.

METHODS

In some experiments unanesthetized rats were given 5% sodium chloride by tail vein and allowed no access to food or water until autopsy; in others, rats were given 1.5%, 2.0% and 2.5% sodium chloride instead of water for 2-week periods, which were run consecutively. At the end of the 6 weeks, the colony was divided; the pituitaries from one-half were studied histologically, the others were assayed for anti-diuretic potency. All the pituitaries were weighed in tared bottles. Those to be stained were fixed 24 hours in Bouin's solution, sectioned at 7 μ , serially mounted and stained by Masson's trichome stain (Foot, '33), by which the chromosomes are stained with iron hematoxylin. To prevent washing out of "loose cells", the mounted sections were coated with celloidin and allowed to dry before hydrating and staining. Every fifth section was examined under oil immersion for the number of mitoses, the number of cells in a given area, and the nuclei of the cells in the area were measured by means of a micrometer. A mitotic figure was not considered such unless the nuclear membrane was absent and multiple chromosomes present.

The pituitaries to be assayed were ground together in 0.25% acetic acid (Nelson and Munch, '25) with sea sand, boiled to destroy enzymes present and frozen until ready for injection. They were then thawed, filtered and diluted with distilled water so that one rat pituitary was contained in 5000 cc. Thus 5 cc. of this 1:5000 solution is equivalent to 1/1000 of a rat pituitary. The potency was estimated from the anti-diuresis produced by the intravenous injection of 1/1000 of the pitui-

tary into dogs with experimental diabetes insipidus (Hare, Melville, Chambers and Hare, '45).

RESULTS

The pituitaries from the rats injected with 5% sodium chloride showed a 40% loss in antidiuretic potency (fig. 1); the pituitary weight/body weight ratio was, on the average, slightly diminished (fig. 4); but the mitotic activity was not appreciably affected (table 1).

The rats on oral salt intake (fig. 2) showed a gradually increasing water intake and output with a falling specific gravity of the urine. Histologically, their pituitaries showed 58 and 68 mitoses/100 sections

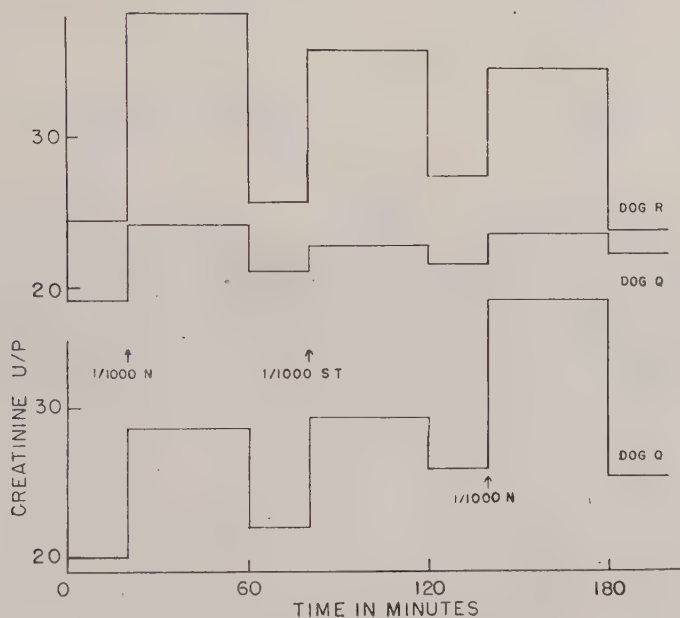


Fig. 1 Assay of pituitaries from rats injected with 5% sodium chloride and autopsied 12 hours later. Creatinine U/P ratio is plotted against time in minutes. Arrows indicate time of injection.

compared to 0 and 4.5 for the controls. The cell population, as determined from counts of selected fields, was only slightly more dense, 13.2 per area, than the normal of 12. Nuclear dimensions averaged 6.0 by 7.7 μ with controls of 6.4 by 7.7 μ . Assay (fig. 3) shows that 1/1000 of a pituitary from the salt treated rats was below the threshold of the dogs used, while a comparable injection of normal rat pituitary produced a marked antidiuresis. The ratio of the pituitary weight to the body weight (fig. 4) is increased.

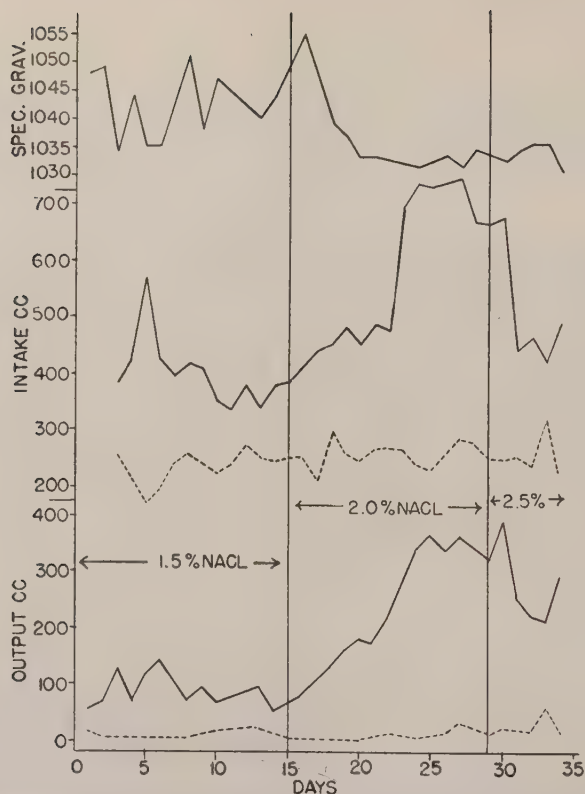


Fig. 2 Water exchange of animals on salt for 6 weeks. Experimental values are represented by solid lines, control values by broken lines. The volume of water drunk and urine excreted and the urine specific gravity are plotted against time in days. The arrows indicate the duration and the concentration of the salt solution provided in place of drinking water.

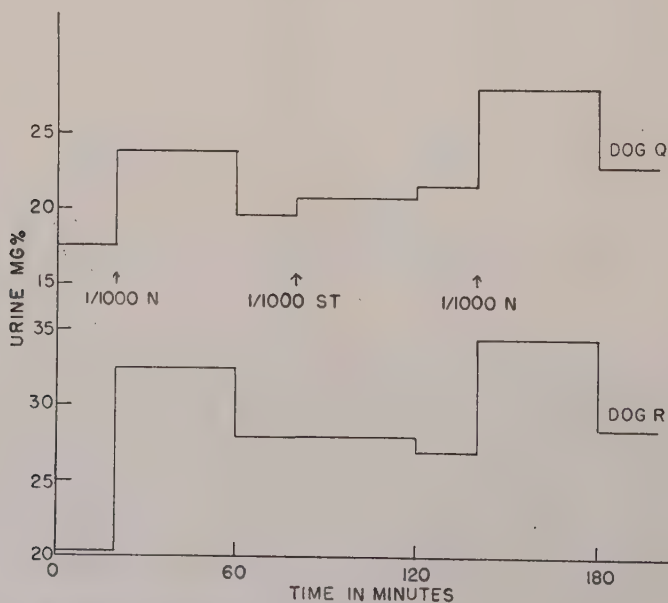


Fig. 3 Assay of pituitaries from rats on oral salt for 6 weeks. Urinary concentration of creatinine in mg. % is plotted against time in minutes. Arrows indicate time of injection.

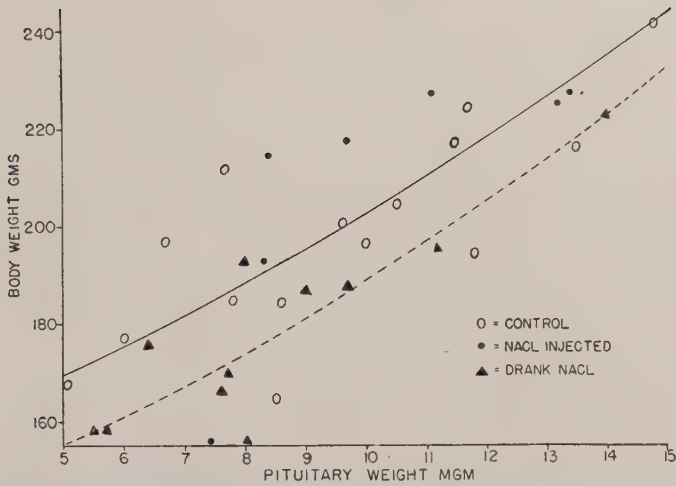


Fig. 4 Effect of salt treatment on pituitary size. Body weight in grams is plotted against pituitary weight in milligrams.

TABLE 1

Tabulation of experimental groups and their treatment. Injection amounts varied according to body weight. Autopsy time is time from start of treatment. Mitoses are expressed as calculated for 100 sections. Antidiuretic potency is expressed in per cent of normal control.

GROUP	TREATMENT	TIME AUTOPSIED	MITOSES/100 SECTIONS	ANTIDIURETIC ASSAY
Experimental A 5 females 5 males	3-6 cc. 5% NaCl IV	6 hours	0	Not done
Control A 5 females 5 males	0	6 hours	0	Not done
Experimental B 7 males	3-9 cc. 5% NaCl IV	12 hours	3.7	60 %
Control B 4 males	0	12 hours	2	100 %
Experimental C 5 females	1.5% NaCl orally 2 weeks 2.0% NaCl orally 2 weeks 2.5% NaCl orally 2 weeks	6 weeks	58	Not done
Control C 4 females	0	6 weeks	0	Not done
Experimental D 8 females	1.5% NaCl orally 2 weeks 2.0% NaCl orally 2 weeks 2.5% NaCl orally 2 weeks	6 weeks	68	Below threshold
Control D 8 females	0	6 weeks	4.5	100 %

DISCUSSION

Either the injection of hypertonic salt solution or the ingestion of salt over a long period decreases the amount of antidiuretic hormone in the posterior lobe of the pituitary. There are no morphological changes in the gross or in stained sections 6–12 hours after injection. In the long treated animals there is so little antidiuretic hormone present it cannot be measured in the injected amount. Here, cytologically, there are increased mitoses substantiating the finding of Selye and Hall ('43). The literature covering the histology of the neurohypophysis gives little information regarding mitoses in pituicytes (Herring, '08, '13; Bucy, '30; Shanklin, '40; Vazquez-Lopez, '42). Shanklin ('44) studied the histogenesis of the pig pituicyte and mentions that mitosis occurs up to the 120-mm. stage. He also shows a photograph of a pituicyte in mitosis with a typical protoplasmic process ending on a vessel. Kerr ('43) found two mitoses in several score mouse pituitaries. The apparent edema of the parenchyma of the neurohypophysis is not borne out by cell counts in a given area, nor is the discrepancy due to a change in size of nuclei. Subjectively there is less colloid than in the injected specimens or controls. The many vacuoles present in the long treated rats appear to be due to the loss of stainable colloid rather than to large numbers of mitotic cells. Many examples of large vacuoles and a small pyknotic nucleus within the same cell are present.

The role of the posterior pituitary in maintaining life during a high salt intake is well presented in the observations of Swann and Penner ('39). They reported that rats with diabetes insipidus died within a week when provided 1.8% sodium chloride to drink. The survival of normal rats on this and even higher concentrations is evidence of the release of pituitrin from the neurohypophysis. While no quantitative measure of the rate of liberation of pituitrin is available, it must be many times normal. The depletion of the pituitrin stores in the pituitary and the increased mitotic activity of the pituicytes result from the prolonged stimulus for increased release of the antidiuretic hormone.

CONCLUSIONS

1. Salt, either by intravenous or oral administration, decreases the potency of antidiuretic hormone in the rat's pituitary.
2. Mitoses appear rarely in the normal pituicyte but about fifteen times more frequently following a long period of salt ingestion.
3. The pituitary increases in size with salt ingestion, but may be normal or smaller than normal 6–12 hours after injection of salt.

4. There are no cytologic criteria demonstrated which indicate the rate of secretion or the amount of pituitrin stored in the neurohypophysis.

ACKNOWLEDGMENTS

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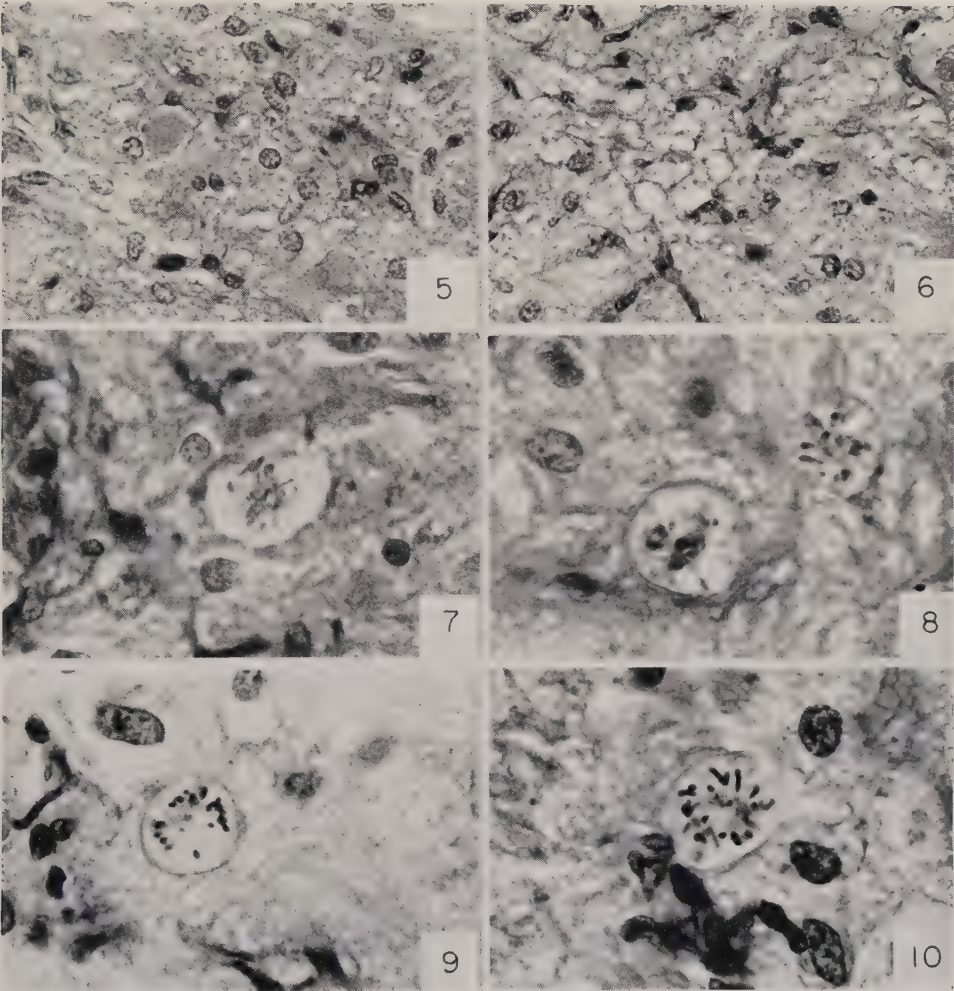
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PLATE 1

EXPLANATION OF FIGURES

All sections were fixed immediately after autopsy in Bouin's solution, sectioned in paraffin at 7μ and stained with Masson's trichrome stain.

- 5 Neurohypophysis of normal rat. High power.
- 6 Neurohypophysis of rat ingesting salt for 6 weeks. There are large numbers of irregular vacuoles. High power.
- 7 Mitotic figure showing shrinkage of the cytoplasm away from the cell wall. Oil immersion
- 8 Two mitotic figures in same field. Oil immersion.
- 9 and 10 Mitotic figures in neurohypophysis. Oil immersion.



A RACE OF HERMAPHRODITE-PRODUCING PIGEONS

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TWO TEXT FIGURES AND THREE PLATES (FIFTEEN FIGURES)

The repeated occurrence of true and pseudohermaphrodites in a strain of pigeons bred in this laboratory was reported briefly several years ago (Riddle and Schooley, '36; Riddle, Dunham and Schooley, '42). More comprehensive information obtained during the past 11 years on the nature and inheritance of the type of hermaphroditism found in this strain is presented in this report.

A strain of Drentisch fowls, said to have been "cultivated by in-breeding" in north Holland during several years, was probably somewhat similar to the present strain. Boring and Pearl ('18) examined and reported upon five abnormal adults of that strain, but there was only one true hermaphrodite in the group examined by them. All of these fowls were regarded as genetic females, although no genetic evidence was provided. In the present strain of pigeons there is satisfactory evidence that the hermaphrodites are genetic males.

The numerous sporadic cases of hermaphroditism, or intersexuality, which have been reported in other species of birds will not be discussed here. Brambell and Marrian ('29) described rather fully the gonad histology of a pigeon which they regarded as in process of sex reversal from female to male. In that case both gonads were ovotestes; the left gonad was enlarged and tumor-like; there was a left oviduct but apparently no vasa deferentia. Nothing was known concerning the ancestry or breeding performance of this bird.

Certain strains of mammals have been found to produce many pseudohermaphrodites along with much rarer cases of true hermaphroditism. Crew ('32) and Eaton and Simmons ('39) have shown that pseudohermaphroditism is inherited in Toggenberg and Saanen goats, and at least one or two true hermaphrodites have been observed in those breeds. In some instances male pseudohermaphroditism in man has been shown to appear repeatedly in certain families (Pettersson and Bonnier, '37; Moebius, '35). A general description of the anatomical features of various types of human hermaphroditism and inter-

sexuality is given by Young ('37). In different species of *Drosophila* hereditary types of intersexes have been obtained and analyzed (Sturtevant, '21; Lebedeff, '39; Dobzhansky and Spassky, '41).

ORIGIN OF THE RACE

One of the strains of pigeons developed at this laboratory, and designated "race 267," produced a true hermaphrodite after about seven generations of more or less intense inbreeding. The bird was functionally male; it fertilized some eggs during the first 2 or 3 years of its life and thereafter became sterile (the normal span of fertility in pigeons is more than 5 years). Race 267 was derived from a mixture of the Jacobin and Fantail breeds and common mongrels. The largest single element in the mixture was Jacobin, and this breed contributed the sex-linked dominant "ash-red" color factor studied by Hollander and Cole ('40). There is no good evidence that the tendency to hermaphroditism is attributable to either the Jacobin or Fantail ancestry; the basis of the defect might have been a sporadic mutation, or perhaps a complex interaction effect. Whatever the source of the abnormal heredity, the males of race 267 had testes of remarkably small size — actually, and in relation to body weight, the smallest of all the breeds or races that have been examined at this laboratory.

Two sisters of the "original" hermaphrodite were outcrossed to males of the Tippler (Tumbler-like) breed. One of these matings produced a true hermaphrodite and the other a pseudohermaphrodite (male with oviduct). Fortunately the ash-red color, by its sex-linked transmission, here permitted the identification of these abnormal individuals as genetic males. When mated with a sister the F_1 true hermaphrodite produced a large number of F_2 offspring; among these were true and pseudohermaphrodites, normal males, and normal females. Chart 1 provides information concerning subsequent breeding procedures, and also indicates the exact pedigree of some of the more important cases of hermaphroditism which were bred or which were permitted to live to maturity. It will be seen that the present stock is derived very largely from the two outcrossed sisters of the "original" hermaphrodite of race 267, though there is a small infusion of stock from other Tippler $\times$ 267 crosses. The Tipplers introduced not only the black (wild-type) allele of the ash-red color but also a recessive sex-linked color factor, "dilution." The present stock is characterized by rather small size (270–370 gm.). We can find no relationship between hermaphroditism and the various color types (other than sex-linked color) which

have been segregated and recombined at random. Except when close inbreeding is attempted, or when certain hermaphrodites are used in matings, reproduction is satisfactory or even prolific.

Complete oviducts practically never occur in males of most stocks of pigeons bred in this laboratory. Two notable cases (weights, 27 and 78 mg.) were found in males derived from a cross between race 267 and Homers. Fragmentary oviducts (usually consisting chiefly of the infundibular portion) are found rather frequently, however, in males of

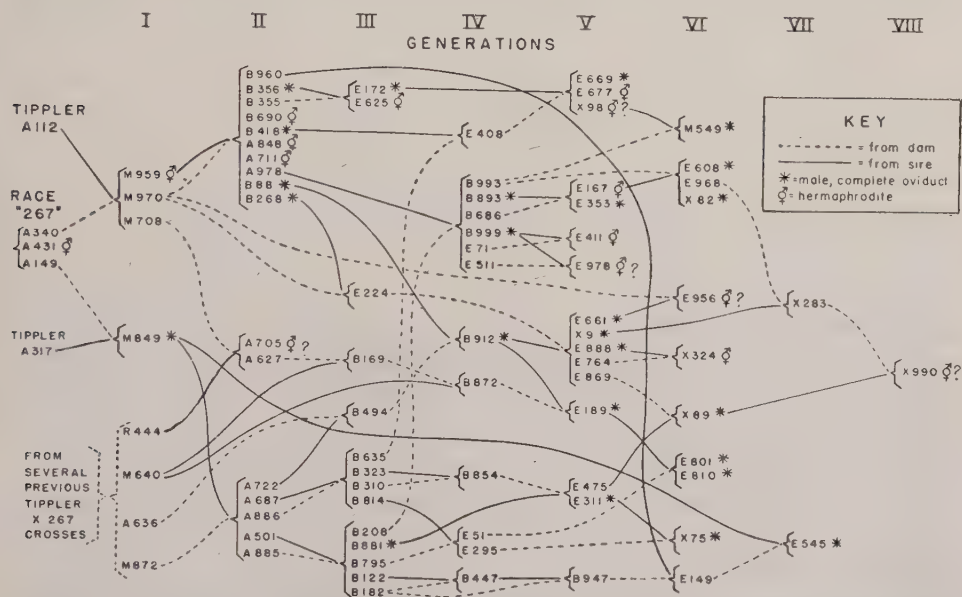


Chart 1 A pedigree network showing the relationships of the more significant examples of hermaphroditism obtained in race T x 267.

Carneau and some other breeds. Oviducal fragments in any breed, like the complete oviducts of pseudohermaphrodites, respond (by growth) to stimulation by estrogenic hormones quite as readily as does the oviduct of a female; the detection of small fragments of oviduct is facilitated by such stimulation. Similar observations have been made on fowls by Juhn and Gustavson ('32) and by Landauer and associates ('39).

ANATOMICAL AND HISTOLOGICAL DATA

Routine observations have been made on the birds of this race by means of laparotomy and at autopsy. Most of the tissues used for histological study were taken from birds sacrificed while in good health.

Such tissues were taken not only from old or adult birds, but from other birds in various stages of immaturity. Not quite all of the gonads which were regarded as ovotestes on macroscopic evidence were subjected to histological study; but, except for three laparotomized adults kept alive to maintain the race, such birds are not listed in table 1. The table provides much information concerning the nature and extent of the abnormalities found in the reproductive organs of adults. A similar table has been constructed from data obtained from specimens dead or killed at various stages of immaturity (from hatching to 8.3 mo.), but publication of that material seems unnecessary. Weights of gonads

TABLE 1

Observations on the more informative hermaphrodites examined in the adult state.

BIRD		AGE AT AUTOP- SY	LEFT GONAD		LEFT OVI- DUCT WEIGHT	RIGHT GONAD		REMARKS
Num- ber	Gen- era- tion		Nature	Weight		Nature	Weight	
		<i>mos.</i>		<i>mg.</i>	<i>mg.</i>		<i>mg.</i>	
A431	0	75	Ovotestis	140	350	Abn. test.	Minute	Fertile *
M959	1	72	Ovotestis	2640	4860	Testis	53	Fertile
A705	2	54	Cystic test.	4120	1900	Flat. test	35	Not bred ¹
A711	2	9	Ovotestis	415+85	1055	Testis	245	Brooded
A848	2	19	Ovotestis	520	1270	Testis	680	Not bred
A978	2	74	Testis	600	121	Testis	1420	Fertile
B690	2	44	Tumor-like	1200	4790	Abn. test.	65	Sterile
E167	5	76	Ovotestis	260	55	Testis	(600)	Fertile
E353	5	18	Testis	1150	100	Testis	1040	Not bred
E411	5	26	Ovotestis	645	141	Testis	275	Not bred
E545	7	6	Testis	675	705	Testis	275	Not bred
E625	3	19	Ovotestis	2510	5435	Testis	485	Not bred
E669	5	17	Testis	770	200	Testis	1000	Not bred
E677	5	17	Ovotestis?	580	1300	Testis	155	Not bred
E801	6	13	Testis	920	106	Testis	1500	Sterile
E810	6	14	Testis	890	102	Testis	1255	Not bred
B999	4	44	Irreg. testis	195	40	Testis	600	Fertile
E956	6	Adult	Irreg. test.?	(600)	(?) ²	Testis	(800)	Alive, fert.
E978	5	35	Abn. testis	(1300)	2301	Abn. test.	1280+86	Sterile
X9	5	22	Abn. testis?	400	44	Testis	450	Fertile
X75	6	14	Testis	605	123	Testis	1510	Not bred
X98	5	Adult	Ovotestis?	(400)	(150)	Testis?	(600)	Alive, fert.
X324	6	33	Three lobes?	(400)	650	Testis	(200)	Sterile
X990	8	Adult	Ovotestis?	(800)	Large	Testis	(800)	Alive, fert.

* Sterile during final 3 years.

¹ Partially thyroidectomized 6 months before autopsy.

² Not seen in biopsy.

which are inaccurate, or which represent estimates made at biopsy, are placed in parentheses.

It seems necessary to provide detailed descriptions of a few of these hermaphrodites. For the histological studies conducted on some of these gonads we are much indebted to Dr. H. H. Dunham.

True hermaphrodite no. A711. At the age of 6.6 months this bird was thought to show interest in a nest and was placed in a separate cage and given a male mate. Five weeks later A711 began to incubate in an empty nest. Males may begin such incubation at the time eggs are due from their mates; and females may do so following ovulation to the body cavity. A711 persisted more or less in incubation until it was killed 5 weeks later at the age of 9.0 months. Proof that the apparent broodiness was real was obtained from the observation that the bird's crop-sacs were much enlarged (weight 3.410 gm., instead of a normal resting value of 0.900 gm.) and actively secreting crop-milk.

Macroscopic examination (fig. 1) indicated testicular masses on right and left sides, and also an ovotesticular mass on the left side. A fairly large left oviduct (1.055 gm.) and a small right oviduct (42 mg.) were present. Vasa deferentia were not identified, but the right vas was probably present. The right testicular mass weighed 245 mg., or little more than one-third of the normal weight of an adult right testis; it was irregular in shape and "lumpy," and definitely harder or denser than normal active testicular tissue. When the gland was cut across at its center the contents did not bulge or flow as in the case of a wholly normal testis. The main part of the testis-like left gonad weighed 415 mg. and was rather like the right gonad in appearance except that several hyaline spots (0.3 to 0.5 mm. in diameter) were visible beneath its surface. Extending posteriorly from this mass was a more slender part whose ventral surface had the appearance of true immature ovary. This ovary-like mass had bulges on its surface indicating the presence of ova, and — together with a dorsal strip of tissue which on section proved to be testicular — weighed 85 mg.; only about one-third (30 mg.) of this structure proved to be true ovarian tissue, and all the ova in this ovotestis would weigh less than 1 mg.

Histological study confirmed and extended the above description. The right gonad (fig. 4) is a well encapsulated testis and contains no ova. The epididymis extended along a very restricted part of this gland, apparently leaving the expanded anterior end of the testis without ex-current ducts; numerous connections with the testis tubules were found, however, and proximal parts of the epididymis were rather well filled

with sperms. In the posterior part of this testis a fair number of the seminiferous tubules seemed normal, and such tubules showed all stages of spermatogenesis and many mature sperms (fig. 5). Anteriorly the relative number of active tubules decreases; that region was filled with irregular, ill-defined tubules showing early stages of spermatogenesis but no mature sperms. Perhaps the lack of an excretory path from the anterior tubules inhibited the production of mature sperms.

The larger and most anterior mass of the left gonad is histologically quite unlike the right gonad. Numerous small ova are found among the very densely packed seminiferous tubules (fig. 2). This part of the

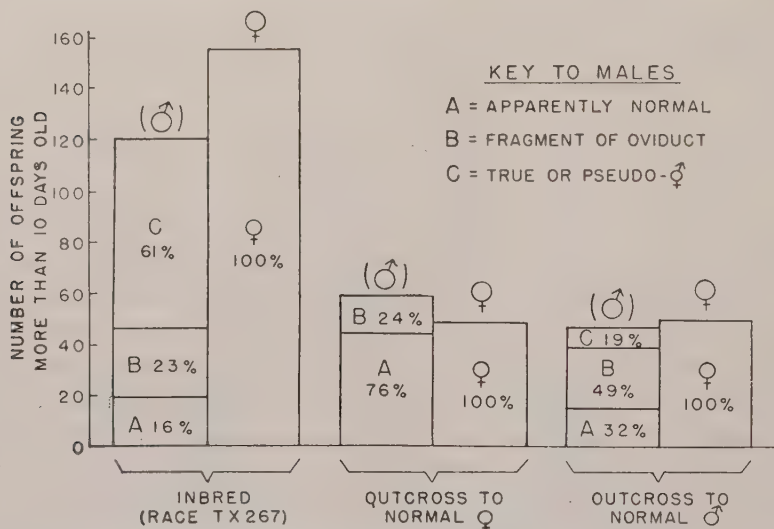


Chart 2 Types and proportions of offspring from three different kinds of matings involving race T × 267. No offspring aged less than 10 days are included.

gonad had no epididymis, therefore no outlet for sperm, and spermatogenesis again seems inhibited; although there are very many mitoses in the epithelium very few sperms are present; in general, the production of spermatids is the end-point of spermatogenesis in this gonad. Posteriorly there are many dilated seminiferous tubules (lower center of fig. 2) whose lumens are filled with germinal tissue (much of which is undergoing phagocytosis), white blood cells and fragmented tissue. These enlarged tubules proved to be the structural basis for the clear spots seen beneath the surface before the tissue was fixed; their history probably includes a stage of overfilling with their own products (no outlet), followed by degenerative changes. Ova up to 200 μ in diameter are found in this anterior segment of the left ovotestis; they are more

numerous near the periphery but are not at all limited to a surface position. These ova are surrounded by a single layer of granulosa and a thin sheath of connective tissue simulating a theca, and are not otherwise set off from the surrounding seminiferous tubules. The ova themselves closely resemble those of similar size in normal ovaries.

Because of the very dense nature of the testes (right and left), and the degenerative changes occurring in them, the differences between tubular and intertubular structures are often indistinct; this condition definitely prevents an estimate of the quantity of interstitial tissue present. Large vesicular cells, much like primordial germ cells or granulosa cells, are abundant in the stroma of the ovarian segment of the left gonad. There is no well-differentiated epididymis on the left side for either segment of the ovotestis, and no connection between the few vasa efferentia present and the seminiferous tubules. It seems probable that the rete tubules have not differentiated to the point of establishing connections between the testis tubules and the vasa efferentia.

A slender posterior (and ventral?) segment of the left gonad is definitely ovarian in structure. The germinal epithelium of the ovary passes over imperceptibly into that of the testis at their juncture, and in that region ova and seminiferous tubules intermingle. The granulosa of most of the follicles is incomplete so that the vitelline membrane comes into contact with the theca at many points; obviously this aspect of the environment of such ova may be expected to limit their further development. The largest class of ova measure about 400 μ in diameter. Since these are the largest ova to be found in the whole of this left ovotestis, and the total amount of ovarian tissue is so small, the size of the oviduct of this bird becomes an item of interest. It would seem that neither the ova nor the definitive granulosa cells adjacent to them are present in numbers sufficient to provide the estrogen necessary to maintain the oviduct at the level actually found. The left oviduct was patent and in both size and histology it was similar to the oviduct of a normal adult female pigeon in reproductive rest.

True hermaphrodite, no. A848. Biopsy and sacrifice at the age of 18.8 months supplied macroscopic indications of a normal testis with normal excurrent ducts on the right side, and a very irregular, somewhat flattened left gonad of questionable nature. A left oviduct (weight, 1.270 gm.), rather larger than that of an adult female in reproductive rest, was present; but no left vas deferens was found. After photographing the reproductive organs in situ (fig. 6) these were closely examined, weighed, and specimens taken for histological study. The right testis

(0.680 gm.) was of nearly or quite normal weight. The abnormal left gonad (0.520 gm.) showed no evidence of ovarian tissue on gross examination, but its "lumpy" and flattened condition (in addition to the presence of a large oviduct) made microscopic study necessary.

Histologically the right gonad (fig. 7) showed normal tubular development, with spermatogenesis proceeding to complete sperm formation. The vasa efferentia contained quantities of sperms; and since the right vas deferens was complete and patent these sperms could reach the cloaca normally.

Serial sections show that the left gonad was attached along only one-fourth of its length, the anterior half and the extreme posterior quarter being free. Within the hilum the vasa efferentia are present in unusual numbers (fig. 8); such large numbers of persistent mesonephric tubules are more common in the bird ovary than in the testis. Some of the vasa efferentia contain masses of sperms; but since there was no left vas deferens there was no outlet for these sperms. Many of these tubules showed degeneration and phagocytosis. The seminiferous tubules near the hilum show considerable spermatogenic activity with the formation of mature sperms. Throughout most of the gland, however, the tubules are nearly solid and spermatogenesis is generally halted before the spermatid stage is reached. The absence of an excurrent path from these tubules was probably associated with this check to sperm formation in a bird that had lived a full year of adult life. Scattered among the seminiferous tubules are ova up to $200\ \mu$ in diameter (fig. 9). They are situated peripherally and are most numerous in the medial region. Atretic follicles (up to $300\ \mu$ in diameter) are found but the follicular epithelium has only one layer of cells. No ova beyond the primary oöcyte stage are present.

The left oviduct of this bird (like that of no. A711) presents an appearance essentially normal for the adult female whose ovary has functioned but currently contains no ovum in the final spurt of growth; its epithelium, glands and musculature were well developed (fig. 10) and the lumen was open throughout. Apparently this oviduct was perfectly capable of delivering an egg to the cloaca.

True hermaphrodites nos. A903 and A939. These birds (clutch mates) were killed at the age of 2.0 months (6 weeks after hatching). They were similar in having very small juvenile right testes (5.3 and 3.6 mg., respectively) and small left ovotestes (4.7 and 4.4 mg., respectively). Both birds had left oviducts (15.6 and 31.6 mg.) and right and left vasa

deferentia. The testicular tubules in both right and left gonads were relatively quite small (fig. 11). The ovarian tissue extended one-third to one-half of the length of the medial surface of the left gonads.

Miscellaneous cases, nos. E978, X3 and an embryo at hatching. No. E978 was shown by sex-linkage of the ash-red factor to be genetically male. When used as a male this bird was sexually indifferent for a period, but eventually the behavior became somewhat masculine. He failed to fertilize any eggs of three successive mates, though he very dutifully incubated the numerous eggs laid. At autopsy the reproductive organs were found to consist of three irregular gonadal masses lying transversely rather than longitudinally in the body. A large left oviduct and a small posterior fragment of a right oviduct were present. Two vasa deferentia were found, but they were not convoluted and no sperm was present. Histological sections of the three gonad masses revealed a general testicular structure, but the tubules were very irregular and there was no active spermatogenesis and no sperms. A number of cystic (apparently degenerating) regions were present, but probably no ova were observed in the parts of these large gonads which were actually studied. The oviduct (2.301 gm.) was histologically similar to that of a female in active reproduction.

No. X3, aged 4.4 months, and a sib of E978 described above, had apparently normal testes and an oviduct weighing 36 mg. (fig. 15). Many individuals of this race — and all of the immature pseudohermaphrodites — are essentially similar to this case.

A cross section of the left gonad of an embryo at hatching is shown in figure 12. In this exceptional case the gonad consists of approximately equal parts of ovarian and testicular tissue. The appearance of these tissues under higher magnification is shown in figures 13 and 14. This testis showed relatively greater development of ovarian cortex than was ever observed in an embryo or adult of this race; in none of the male embryos studied by Lahr and Riddle ('45) was ovarian tissue equally prominent. It therefore seems probable that this bird would have remained a true hermaphrodite when mature.

INHERITANCE

It was noted earlier that a fair proportion of the true hermaphrodites, and practically all of the pseudohermaphrodites, are capable of breeding as males, at least temporarily. Seven matings involving five probably true hermaphrodites (M959, B999, E167, X98, and X990)

have been studied with some care, and the following records (total progenies) obtained:

Total eggs (104 with M959 as mate)	217
Eggs lost, broken or infertile (39 with M959 as mate)	46
Dead embryos or squabs, no sex record (19 with M959 as mate)	32
Offspring killed or dead from hatching to 3 days old	16
Normal (?) males	7
Males with oviduct, or ♀	3
Females	6
.....	10
Offspring killed or dead, from 3 days to 3 months old	45
Normal (?) males	7
Males with oviduct, or ♀	16
Females	22
.....	23
Offspring killed or dead, over 3 months old	78
Normal (?) males	6
Males with fragment of oviduct	3
Males with complete oviduct, or ♀	28
Females	41
.....	37

Fertility and hatchability were normal, except for the later years in the breeding of ♂₊M959. From the above results it appears that a close approach to the normal 1:1 sex ratio is obtained only if the abnormal individuals are classed as genetic males. Evidence obtained from the inheritance of sex-linked color factors has also indicated that these hermaphrodites are genetic males. Presumptive females from such sex-linked matings have always proved at autopsy to be normal females, while presumptive males have often been abnormal. Again, in the breeding tests the hermaphrodites have proved heterozygous or homozygous for the color factors, like normal males, while females have always been hemizygous.

Although the hermaphrodite-producing race had not been selected and inbred to the point of uniformity, it was considered desirable to make some outcrosses in order to obtain information on the inheritance of the trait. Accordingly, reciprocal outcrosses were made to normal stock (Tipplers excluded). Most of the "normals" employed were Homer-Run crossbreds.

Two hermaphrodites (E167, X98) and four pseudohermaphrodites (E172, E956, X82 and X292) were successful in producing F₁. X292, not included in table 1, was a nephew of E801 (see pedigree chart). From a total of 226 eggs the sex records were obtained for 132 offspring. There were thirty-five additional embryos (or decomposed dead young) whose sex was not determined. Of the 132 recorded for sex a group of 106 were

at least 10 days old; among these, forty-eight were females, forty-four were apparently quite normal males, and fourteen had fragmentary oviducts. The twenty-six remaining offspring were 3 days old or less; fourteen of these were females and twelve were apparently normal males. A summary of these results is given in chart 2.

The occurrence of males with fragmentary oviducts was noted among the offspring of all the birds listed above except E172. The frequency of this occurrence may be a little greater than in a normal population, and in our opinion it is definitely greater than in the parental population of Homer-Runt crossbreds though an accurate control series was not obtained. It may be concluded that if departure from normal exists it is not at all impressive. This result, moreover, cannot be attributed to lower fertility of the hermaphrodites used; ♂E167 produced the second largest number of young. It is clear that the results are quite different from those obtainable from these birds when mated to females of their own race.

In the reciprocal outcross ten females of the hermaphrodite-producing race were successful in producing F_1 ; X334 and X833 (sisters of X324) and also B993 and E408 were among the females used; the other six females had no outstanding history and may be considered a random selection. Most of these matings showed presumptive sex of offspring by means of sex-linked color factors. A total of 215 eggs were laid and sex records were obtained for 154 offspring. Of these, ninety-six were more than 10 days old, thirteen were aged 3 to 10 days, and forty-seven were younger than 3 days. The findings in all of the three age-groups are similar: males were rarely normal, and a considerable proportion (19%) had complete left oviducts. Probably no true hermaphrodites were obtained; in the youngest birds, however, this condition would not always be detected. In no case did the sex-linked color factors indicate a complete reversal of sex.

The striking difference in the results of the reciprocal outcrosses merits careful consideration. X334 and X833 were unusual since they produced practically nothing but normal males and females; and X334 continued this record even with a second mate. All other females produced one or more sons with fragmentary oviducts, and four of them produced some sons with complete oviducts. It is our opinion that X334 and X833 were quite normal and not typical of this race.

For purposes of comparison with results from the outcrosses the data obtained from thirty matings within the race were selected at random. All of these matings ran concurrently with the outcrosses and the off-

spring were examined with equal care. In chart 2 results for all offspring aged more than 10 days are given in graphic form. It is obvious that results from the reciprocal outcrosses differ from each other, and that neither result agrees with that obtained from matings within the race. The differences are clearly significant, statistically. Is there a sex-linked factor responsible for the development of the oviduct in the male? This possibility seems to be excluded by the fact that it will not fit the results obtained from the outcrossed hermaphrodites. A sex-linked factor would necessarily be dominant to normal, and the hermaphrodites would have to be considered at least heterozygous; not a single offspring, however, was in the pseudohermaphrodite class (i.e., no male with a complete oviduct). If not a sex-linked factor, might an autosomal factor be postulated? Here again it would be necessary to assume dominance to normal in order to explain the occurrence of pseudohermaphrodites in F_1 . This assumption is not in accord with the dissimilar results obtained from the reciprocal crosses.

Other possibilities that may be suggested include aberrations of the sex chromosome — such as a duplicated part, a deficiency, or aneuploidy due to translocation. None of these seem to fit the data. Duplication, as an explanation, would require the assumption that the hermaphrodites are presumptive females; and in that case the sex ratio should be low on the female side. The latter is not the case, and the evidence from sex-linked factors contradicts this interpretation. A deficiency in the sex chromosome seems ruled out for the same reasons cited above against a sex-linked dominant factor; moreover, a deficiency would be expected to be lethal in the female and the homozygous male. Finally, if a translocation were involved a rather high embryonic mortality would be expected; actually the race shows a mortality rate essentially similar to that of normal races. Thus, no purely genetic explanation fits the data.

A maternal non-genic inheritance effect was observed in *Lymantria* by Goldschmidt ('34). It is both useful and possible to state specifically our conception of the "non-genic maternal effect" which supplements genic action and resolves the difficulties presented by the breeding tests. That view is fully presented later (see Discussion). Here we note only that excess of estrogenic hormone in eggs has been observed to feminize male embryos of fowl and doves to a greater or less degree; and that it seems highly probable that the females of the hermaphrodite-producing race deposit unusually large quantities of estrogen in some or many of their eggs.

Obviously, however, genetic factors cannot be excluded from the production of this abnormality. There must be a genetic basis for the assumed hypersecretion of estrogen. And if estrogen alone were the cause of hermaphroditism in this race, one would expect similar types and proportions of offspring from females of this race regardless of whether they were mated with males of their own or of another race. The data show that this is not the case: more hermaphrodites and pseudohermaphrodites are produced when cross breeding is avoided. The initial cross with the Tippler provides a definite and interesting exception, and it is our opinion that at least some Tipplers carry a genetic factor which favors the expression of this trait. Our stock of Tipplers frequently have testes of somewhat irregular shape, and it is possible that Tipplers may have a lower threshold to the feminizing effects of estrogen than other normal races.

At present we are unable to provide more precise information concerning the genetic basis of this type of hermaphroditism. There may be one only or several factors involved. The small size of the testes in race 267 and the irregular shape of the testes in the Tippler may reflect the action of the same or of different genetic factors, and both races seem to have contributed something which favors the feminization of the reproductive organs of males. In addition, the breeding evidence points to a non-genic maternal effect on the sons, and especially on those sons inheriting a predisposing constitution.

DISCUSSION

Approximate or partial understanding of these cases of true and pseudohermaphroditism in genetic males may be obtained from a consideration of facts which show (a) that an ovotestis (left) exists as a "normal" condition in a stage of the embryonic development of the testes of several (perhaps all) birds and reptiles, (b) that this condition exists in normal pigeons, and indeed in a more accentuated form in this race of hermaphrodite-producing pigeons (Lahr and Riddle, '45), and (c) that the amount of ovarian tissue temporarily present on the embryonic left testis of doves was increased and made to persist (and oviducts made to develop) by estrogen injected into mothers having an egg (yolk) in a suitable stage of development (Riddle and Dunham, '42).

The more important literature on this subject was reviewed by Lahr and Riddle, who made a histological study of ovarian cortex on the left testes of ordinary breeds of pigeons and compared those results with similar observations made on this race of hermaphrodite-producing

pigeons. In the former case ovarian tissue is present on left testes (not observed on right testes) during most of the final third of the incubation period, but in such birds it has completely atrophied at hatching. In most (possibly all) males of the hermaphrodite-producing race this ovarian cortex tended to persist somewhat longer than in normal races. It seems obvious that in the case of true hermaphrodites this ovarian tissue persists from the embryonic period into and through the adult life. Moreover, since eggs provided with extra estrogen by ring dove mothers produced intersexual males which were not markedly different from the present series of hermaphrodites, it seems probable that extra estrogen — presumably present in the eggs of this race — constitutes a part of the mechanism by which hermaphroditism is made to persist from the embryonic period into adult life. Riddle and Dunham further concluded that "it is probable that much or all of the evanescent ovarian cortex normally found on the embryonic testes of birds and reptiles arises not through genic action but through action of estrogen." In this special race, however, genetic constitution is also involved in the maintenance of hermaphroditism.

The frequent occurrence of testes in which a large portion of the gland was abnormally dense or firm is an impressive fact. We suspect that such conditions led, after weeks or months, to resorption of whole areas of these testes; perhaps the narrow connection between epididymis and testis, and the frequent failure of testicular tubules to connect with efferent ducts, are involved in those changes. If such changes actually follow the development of "hardened" areas this might account for (a) some of the general diminution of testis size in this race, and (b) for the very small size of the gonads of the "original" hermaphrodite (A431). It is our opinion that only the study of a series of these gonads by a competent pathologist could determine the consequences of the formation of these "hardened" areas in testes.

The size relationship of the right and left testes of the birds listed in table 1 is quite abnormal. Earlier data of Riddle ('18) show that in adults of normal pigeons the right testis is usually 5 to 35% larger than the left; in some cases the left testis is larger than the right by comparable percentages. In thirteen of the twenty-four pairs of gonads listed in table 1 a difference exceeding 100% is found; and in four cases the weight of one gonad was 10- to 50-fold greater than the other. This extraordinary disruption of the usual size relationships found in the testes of males of normal breeds must be regarded as one of the most marked of the anatomic distortions which characterize the hermaphrodite-producing race.

It is notable that many of these hermaphrodites, including some pseudohermaphrodites, have shown the wide separation of the pubic bones which especially characterizes the adult female pigeon. This separation of the pubic bones is probably a specific response to estrogenic hormone; it therefore suggests, as do enlarged oviducts, that many of these pseudohermaphrodites produce notable amounts of estrogenic hormone. A further consideration of hormone production and other constitutional elements in this race is given elsewhere by Riddle ('45). It was there noted, for example, that although many such birds failed to do so, one pseudohermaphrodite gave practically conclusive evidence (through large increase of plasma fat) of ability to secrete relatively large amounts of estrogenic hormone.

When adult female pigeons cease to produce ova above 4.5 mm., as in hypophysectomy, the oviduct diminishes within less than 10 days to about 0.459 gm. (average of 15 cases; unpublished data). In normal female pigeons the periodic increase and decrease of granulosa cells within the ovary seem, roughly at least, to parallel the increase and decrease in oviducal weight. Certainly, however, no such relationship of definitive granulosa cells to oviducal size existed in the case of A711, A848 and of some other hermaphrodites (of both types) examined. This fact supplies two indications: First, that ovarian tissue other than granulosa produces the estrogenic hormone in birds. Though this is the view commonly held by most workers it can not be said to have been established. Second, either the ovarian endocrine function of the ovarian parts of these ovotestes was being continued in the absence of active growth of ova, or the testes of these hermaphrodites were producing estrogenic hormone; or finally, oviducts of these hermaphrodites — unlike normal ones — are capable of stimulation by male hormone (or by a modified male hormone) produced by the rather active testes of these birds. These alternatives are mentioned both to suggest them as near or remote possibilities, and to indicate that at present the large size of oviducts found in adults of both true and pseudohermaphrodites poses a challenging problem.

SUMMARY

A case of true hermaphroditism occurred in race 267 and the trait was perpetuated in a cross between a sister of that hermaphrodite and a Tippler pigeon (called race $T \times 267$). The race is still maintained after more than eight generations.

Progeny in this strain consist of normal females, normal males, males with oviducts which vary from fragments to nearly 1% of the

body weight (pseudohermaphrodites), and birds with a right testis and a left ovotestis and complete oviduct (true hermaphrodites).

Both types of hermaphrodites are shown to be modified genetic males, and most true hermaphrodites are fertile when bred as males.

Males of the race (267) in which this trait arose were characterized by unusually small testes, and those of the Tippler race by irregularly-shaped testes. Both races seem to have contributed something which favors the frequent appearance of the trait in offspring.

Reciprocal outcrosses of males and females of this race to normal races produce unequal types and proportions of sexually modified offspring, and breeding within the race produces the highest proportion of modified offspring. The results indicate that one or more genetic factors together with a maternal non-genic influence is involved in this intersexuality of some of the male offspring.

The non-genic influence is tentatively identified as a production of unusual quantities of estrogen which, after inclusion in many or most eggs, causes an increase and persistence of ovarian cortex on embryonic left testes as earlier observed by Riddle and Dunham.

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EXPLANATION OF PLATES

The figures are unretouched photomicrographs. The tissues were fixed in Bouin's fluid and stained with haemotoxylin and eosin with the exception of that shown in fig. 11 which was fixed in Champy and stained with acid fuchsin and methyl green.

Key to symbols: Ht, heart; K, kidney; L Od, left oviduct; OT, ovotestis; T, testis; Ov, ovary; R Vd, right vas deferens.

PLATE 1

EXPLANATION OF FIGURES

- 1 General view of reproductive system of hermaphrodite pigeon, A711. Natural size.
- 2 Low power view of the left ovotestis of A711 showing two ova among testicular tubules. Relatively few sperms but many spermatids in this gonad. Ova are not limited to the peripheral position. $\times 45$.
- 3 Low power view of the junction between ovotestis and ovary in left gonad of A711. The tunica is practically continuous around the ovotestis. The left epididymis does not contain sperms but some sperms are present in adjacent tubules. The granulosa of many ova is incomplete. $\times 45$.
- 4 Low power view of junction of the right testis with its epididymis in A711. This testis is well encapsulated and contains no ova. Some tubules show all stages of spermatogenesis but only a small number are producing sperms. The epididymis extends along only the narrow posterior part of the testis and contains normal sperms. $\times 45$.
- 5 High power view of a section through the right testis of A711 showing details of germinal epithelium in an active tubule. $\times 300$.

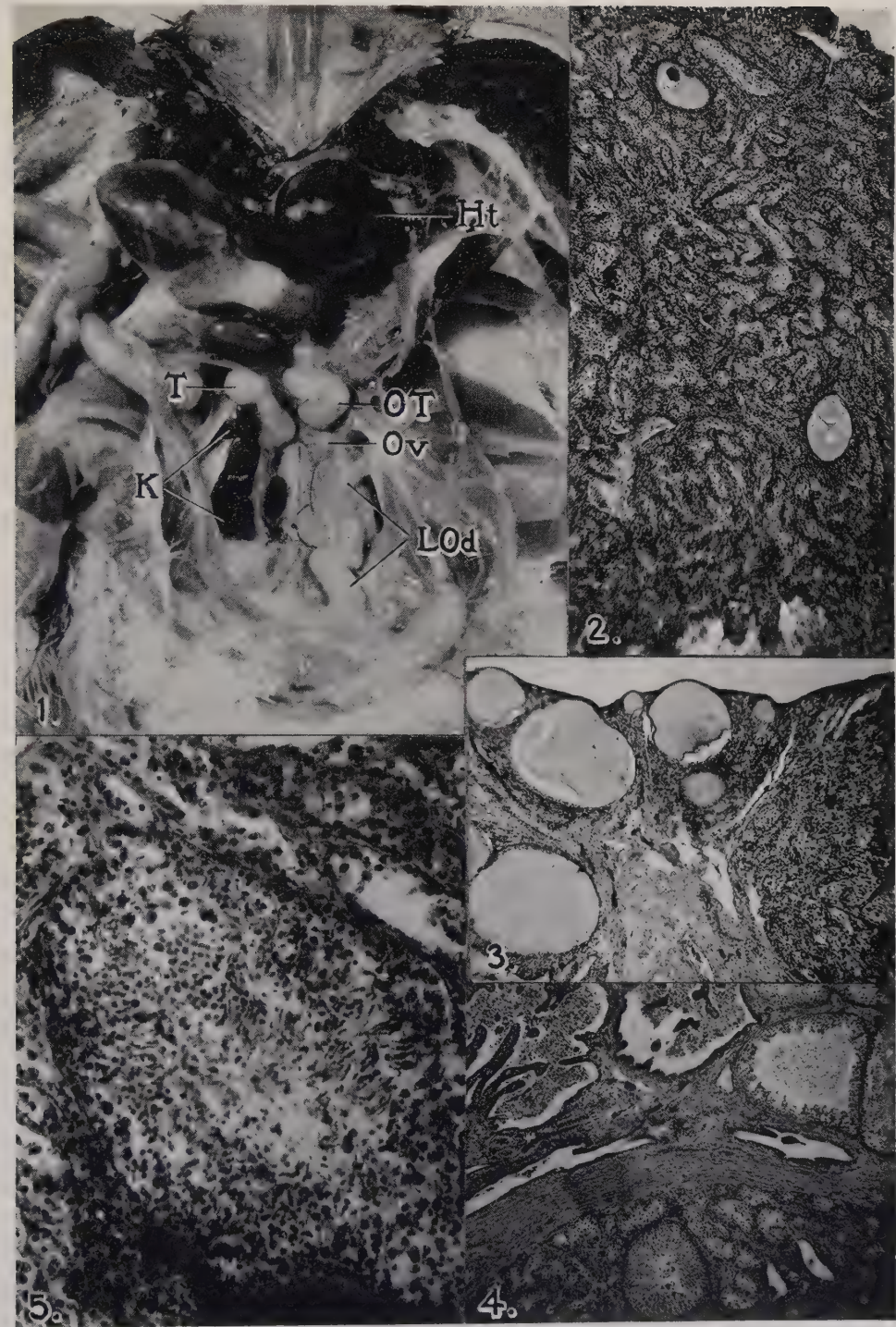


PLATE 2

EXPLANATION OF FIGURES

- 6 General view of reproductive system of hermaphrodite pigeon, A848. Natural size.
- 7 View through right testis (A838) and epididymis where sperms are present. $\times 72$.
- 8 View of left gonad (A848) and vasa efferentia. Sperms are present in the efferent ducts. $\times 72$.
- 9 Section through the left gonad (A848) showing ova, which in this gonad are mainly peripheral, and adjacent tubules. $\times 72$.
- 10 Section through left oviduct of A848 showing well-developed epithelium and musculature. $\times 72$.

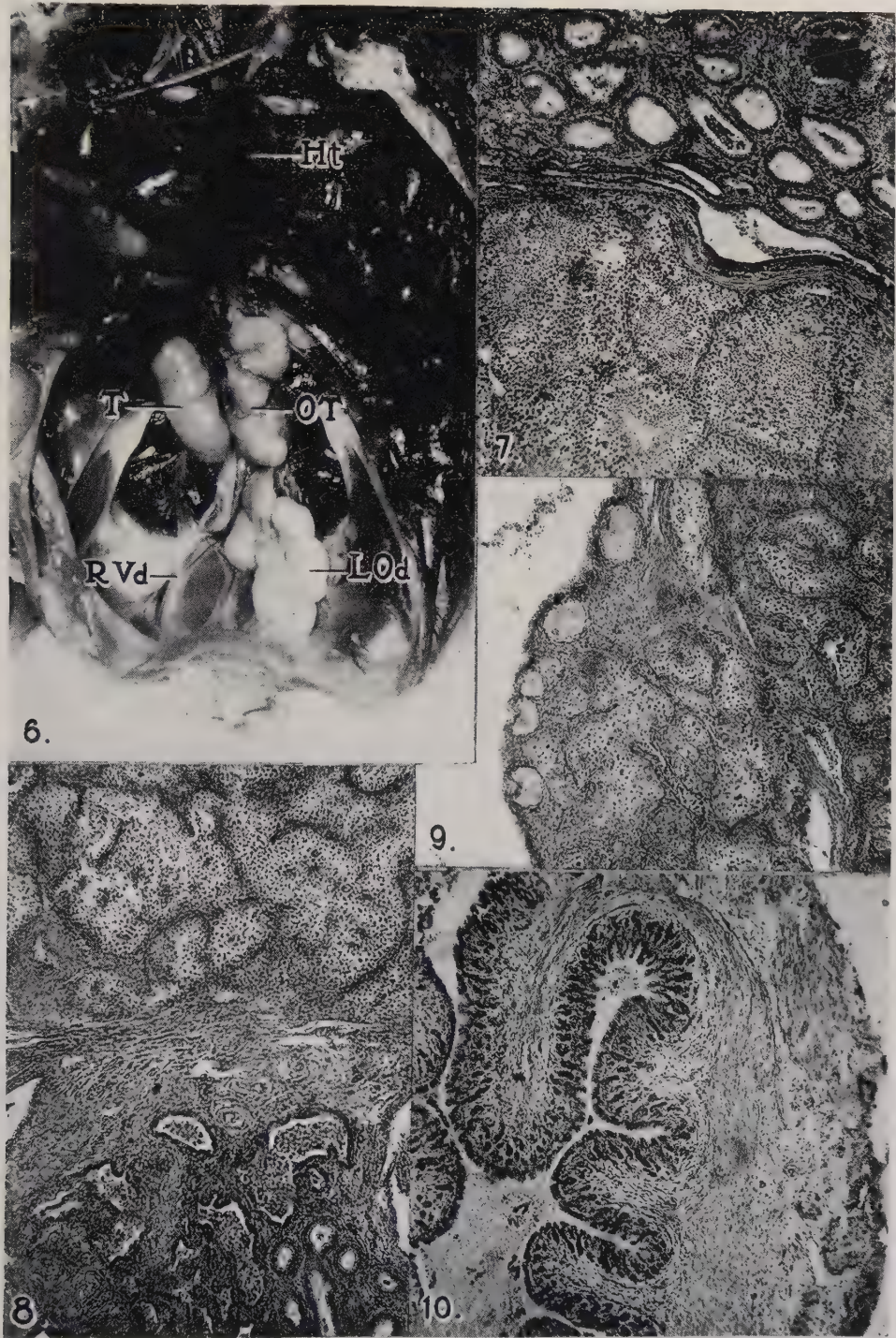


PLATE 3

EXPLANATION OF FIGURES

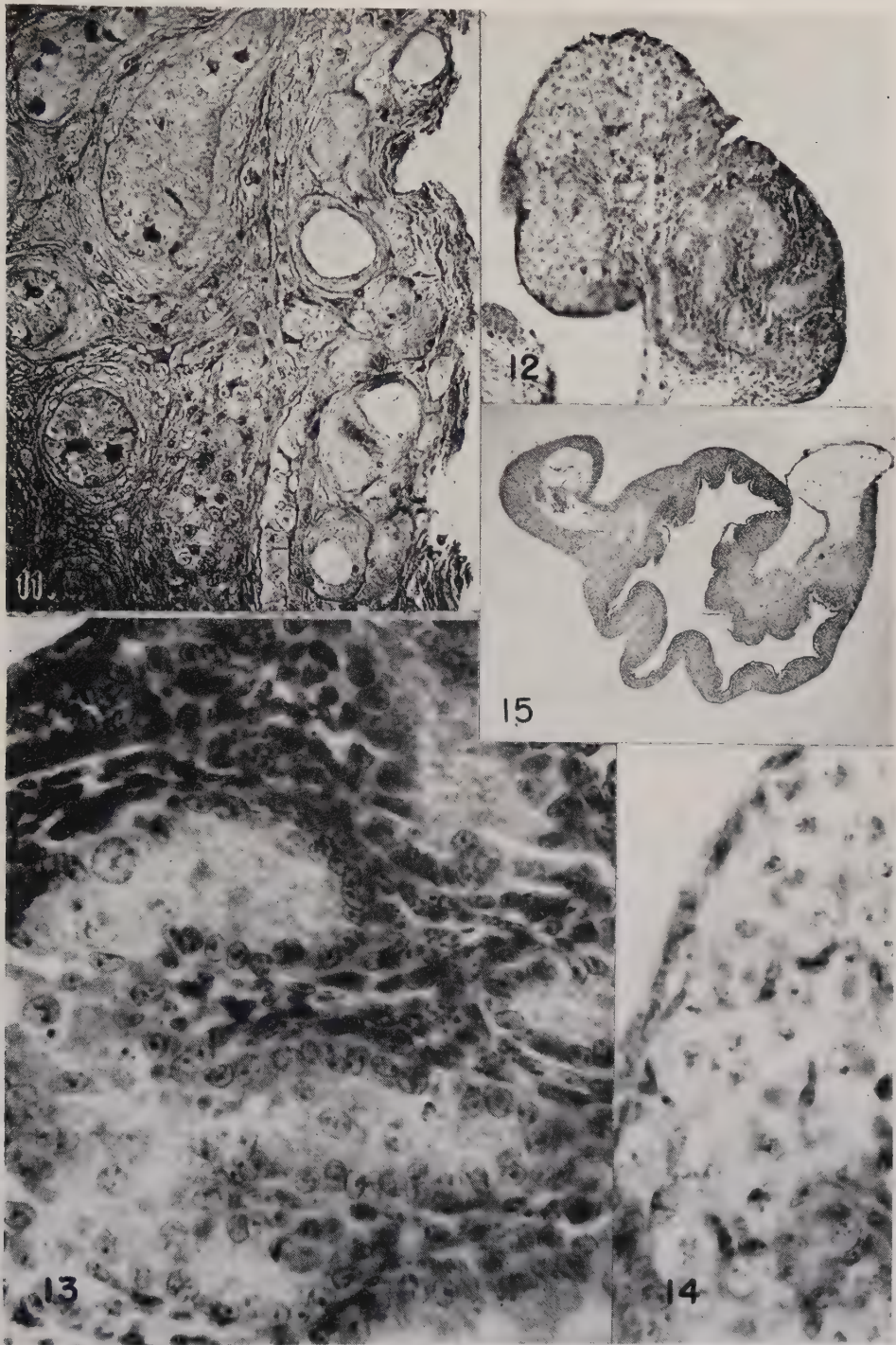
11 Sectional view of ovotestis of A939, aged 2.0 months. The stroma of ovary and testis intermingle at many points. $\times 72$.

12 Low power view of left ovotestis from an embryo at hatching (sib of E608, sixth generation). The amount of ovarian cortex (at left) greatly exceeds that ever observed on a left testis of a normal male embryo, and is also the largest amount observed in an embryo of the hermaphrodite race.

13 Higher magnification of testicular portion of figure 12 showing tubules. $\times 300$.

14 Higher magnification of ovarian portion of figure 12. $\times 300$.

15 Section of left oviduct (wt. 36 mg.) of ♂X3 (sib of E978, fifth generation) aged 4.4 months. The testes were apparently normal, weights (right) 195 and (left) 190 mg. $\times 34$.



INTERSEXUALITY IN MALE EMBRYOS OF PIGEONS

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ONE TEXT FIGURE AND ONE PLATE (SEVEN FIGURES)

A transient intersexuality in male embryos of certain species of birds has been reported. The species in which this condition has been observed include the fowl (Laulanié, 1886), English sparrow (Witschi, '35), blackbird (Amsel) and pheasant (Unger, '36, '37), hawks (Stanley, '37) and ring doves (Riddle and Dunham, '42). This anomaly involves a temporary development of ovarian cortex on the left testes, but usually not on the right testes, of genetic male hormones. The purposes of the present study was to learn whether this type of intersexuality exists in the pigeon, and also to compare the conditions found in normal breeds of pigeons with those existing in a special strain of hermaphrodite-producing pigeons. The existence of a strain of the latter type was briefly reported by Riddle, Dunham and Schooley ('42), and completed studies on this race were published elsewhere (Riddle, Hollander and Schooley, '45; Riddle, '45).

MATERIAL

The embryos were obtained from various races bred in our own colony. The races considered "normal" included Carneaux, Kings, Homers, Tipplers and mixed or hybrid types. The females of the hermaphrodite-producing strain are normal; the genetic males, however, are of three types — normal, pseudohermaphrodites (with oviducts) and true hermaphrodites with left ovotestes (Riddle, Hollander, Schooley, '45). Daily records on all breeding birds enabled us to know the exact age of all embryos. Although the two eggs of the pigeon's clutch are laid about 45 hours apart the "first" egg usually hatches less than 24 hours in advance of the "second" egg. The actual age of embryos from "second" eggs was known and used, and embryos from "first" eggs were regarded as 1 day older than those in corresponding "second" eggs. A total of sixty-one left testes of embryos ranging in age from 14 to 23 days (5 days after hatching) were studied.

With the aid of binocular magnifiers the left gonad was dissected from the male embryos soon after taking the egg from the nest. The gonad was fixed in Bouin's fluid, embedded in paraffin and sectioned at a thickness of 6 to 8 microns. The sections were then stained in hematin or Mallory's triple stain; the latter, although a poor nuclear stain, was used in most cases because it gave a better differentiation between ovarian (cortical) and testicular tissues. The entire gonad was sectioned serially and each section carefully examined for the presence of ovarian cortex.

RESULTS

Sex differentiation has progressed sufficiently in pigeon embryos of 14 days to permit the identification of sex by macroscopic observation. The sex of younger embryos is less readily identified by gross observation. For this reason, together with the fact that ovarian cortex was always found on left testes at this stage, embryos aged 14 days were the youngest used in this study. Six right testes of embryos aged 14 to 18 days (hatching time) were studied and found to be entirely free of cortical tissue.

A thin but well-formed tunica albuginea was present in all 14-day embryos. At this and later stages it enclosed the testicular portions and assisted the location and identification of cortical tissues; the latter were always found outside of the tunica. When cortical cells were present they were most frequently located on the ventral and posterior surfaces of the testis. In a few cases they were found in groups or clusters of a few cells scattered irregularly along the entire ventral surface.

Chart 1 presents the main results of the study in graphic form. Histological examination indicated little or no change in the volume of ovarian cortex present at days 14, 15 and (in the hermaphrodite race at) 16 days. The graph shows that the cortex was found much less frequently in 17-day embryos of normal breeds of pigeons, and that in those breeds it had disappeared completely at the time of hatching (see also figs. 3, 4 and 6). In embryos of the hermaphrodite-producing strain, however, ovarian tissue was present in all embryos of 14 to 16 days (fig. 7), and also present on the testes of a majority of the 17-day and 18-day embryos. There was no reason to believe that these embryos were retarded in their development, and it is known that embryos of this strain regularly hatch at the expected time (18 days of incubation). In five of seven embryos aged 17 days cortex was present, and at 18 days (hatching) three of four embryos retained cortical tissue (fig. 1). At

these later stages the condition of the cortical cells varied more than at earlier stages. In some cases degenerative changes were definitely indicated by a thinning of the epithelia and by the presence of pyknotic nuclei. In other cases the epithelia were highly developed and the cells seemed normal (fig. 5). In three of six testes taken at 5 days after hatching (23 days) cortical tissue was still present. Presumably, occasional ovotestes of this type persist into adult life and thus become the true hermaphrodites which this race is known to produce.

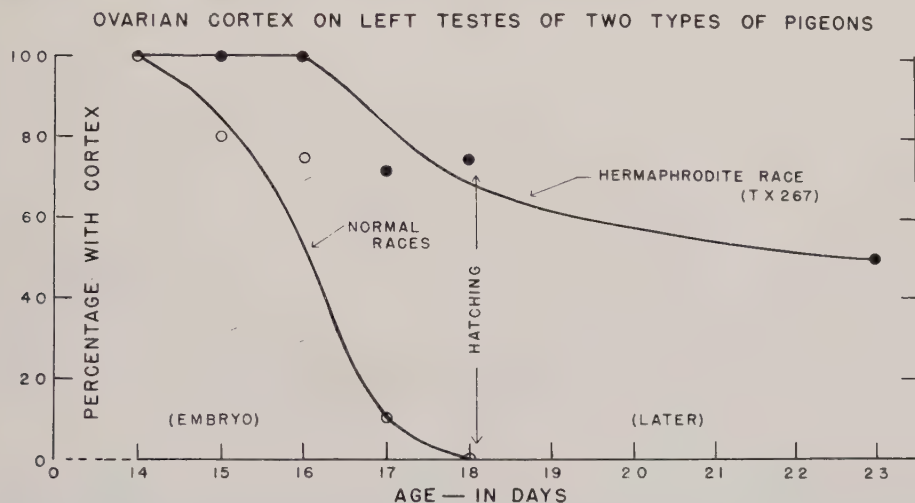


Chart 1 Showing, in embryos of two types of pigeons, the percentage of left testes on which ovarian (cortical) tissue was present. The number of testes of normal type examined at various embryonic stages was: 5 (14 days), 5 (15 days), 8 (16 days), 10 (17 days) and 5 (18-19 days). The corresponding numbers for the hermaphrodite type (T x 267) were: 3 (14 days), 3 (15 days), 5 (16 days), 7 (17 days), 4 (18 days) and 6 (23 days). Ovarian tissue degenerates more slowly on testes of birds derived from the hermaphrodite-producing race than on testes of birds derived from normal races.

The observed regression of the ovarian cortex was accomplished through atrophy or degeneration of the cortical cells. The process was most evident in the 16- and 17-day-old embryos from normal races of pigeons. Similar degenerative changes were evident in a smaller proportion of hermaphrodite-strain embryos of the same or slightly older age groups; this observation is in accord with the fact that complete disappearance of the cortex prior to hatching was observed in only (approximately) one-third of the embryos of this type. At 5 days after hatching the cortical cells had disappeared in three of the six specimens and in three others the appearance of the cortical cells in embryos of this strain indicated a continued reduction (degeneration) of the corti-

cal tissue. Since adult true hermaphrodites are relatively rare it is not surprising that a maximum development of the ovarian cortex was maintained in neither of the six embryos examined at this stage.

DISCUSSION

Some similar manifestations of intersexuality produced by experimental methods should be noted. A number of investigators have found that the injection of estrogens into hens' eggs in the early stages of incubation result in the production of a modified left testis (ovotestis) and the persistence of the left oviduct (Kozelka and Gallagher, '34; Willier, Gallagher and Koch, '35, '37; Breneman, '35; Dantchakoff, '36; and Gaarenstroom, '40). Those observations were extended by Riddle and Dunham ('42) who found that injections of estrogens into the muscles of female ring doves, at 26 to 34 hours prior to the ovulation of an ovum, promoted intersexuality in the embryo which developed from that ovum; this was expressed by the persistence of ovarian cortex on the left testes and by the development and persistence of left oviducts. It was concluded that the injected estrogen, added to that normally present in the blood stream at and preceding ovulation, was responsible for the development of the female characteristics of these males.

The type of intersexuality which Riddle and Dunham observed in doves following injections of estrogen is at least rather similar to that found in the strain of hermaphrodite-producing pigeons. Thus a basis for this anomaly may rest on the circumstance that females of this strain of pigeons usually produce, and incorporate into their eggs, unusually large amounts of estrogen. Since this characteristic is inherited it would follow that the ability to produce unusual amounts of estrogen is under genetic control.

SUMMARY

Male embryos from normal races and from a hermaphrodite-producing strain of pigeons were used in these studies. It was found that, as in certain other species of birds, the pigeon embryo shows a temporary development of ovarian cortical tissue on the surface of the left testis.

In testes from normal races of pigeons (33 cases) this ovarian tissue degenerated between the fourteenth day of incubation and the end of incubation (18 days); in such testes the ovarian tissue disappeared completely at or before the time of hatching.

Testes derived from embryos of the hermaphrodite strain (28 cases) differed from those of normal type in showing a delay in the time at

which atrophy of the cortical tissue begins; this atrophy was first observed in two of seven cases examined on day 17. This tissue had disappeared in only one of four embryos at day 18, and in only three of six embryos examined at 5 days after hatching. Birds which retain large amounts of ovarian tissue at and after hatching are presumably the ones which have been observed to possess a left ovotestis and (or) a left oviduct in adult life.

Reference is made to the probable rôle of estrogen in the development and variable persistence of ovarian tissue on the left testes of pigeons.

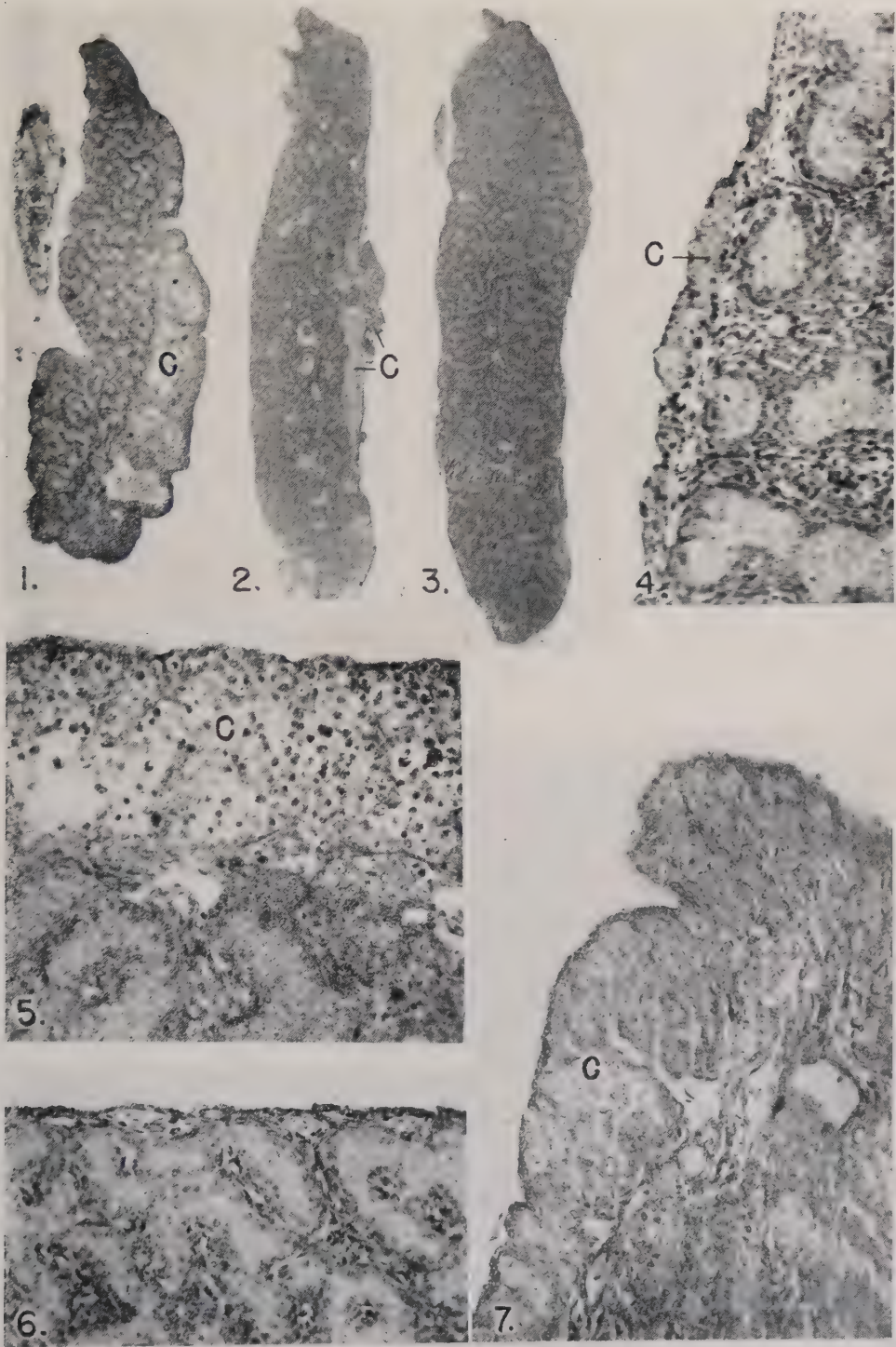
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PLATE 1

EXPLANATION OF FIGURES

- 1 Left gonad (extreme of ovotestis) of a pigeon of the hermaphrodite-producing strain at time of hatching (18-day embryo) showing maximum development of ovarian cortex (C). $\times 40$.
- 2 Left gonad of normal type of pigeon embryo at 15 days of incubation. At this stage some cortex (C) is present. $\times 52$.
- 3 Left testis of normal type embryo of 16 days. No cortex is present in this particular case. $\times 70$.
- 4 Left gonad of normal type embryo of 14 days. Narrow strand of cortical cells shows no degeneration. $\times 210$.
- 5 Higher magnification of figure 1, showing both cortical (C) and testicular tissue. $\times 210$.
- 6 Left testis of normal type embryo of 17 days. Showing tunica albuginea enclosing the testis of a normal embryo of 17 days. $\times 210$.
- 7 A nodule of cortical tissue on the lateral-posterior surface of the left gonad (testis) of a 15-day embryo of the hermaphrodite-producing race. $\times 210$.



ON THE ORIGIN OF THE DIAPHYSIS

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ONE PLATE (SIX FIGURES)

INTRODUCTION

In its most primitive form the diaphysis is represented by a cartilaginous rod, the middle part of which is soon enclosed in a bony tube. The corresponding region of the cartilage is the future center of ossification; at this stage its cells are hypertrophied and are on the verge of being invaded.

This paper describes experiments in support of a thesis advanced for the first time: the bony tube, i.e., the earliest form of the bony diaphysis, appears as a result of an induction phenomenon directed by the growing cartilage.

Before describing the experimental findings, we must first consider the structure of the young growing bone.

STRUCTURE OF THE GROWING DIAPHYSIS

Only those growing extremities which have been used in the experiments will be described, viz., the distal part of the radius and the chondro-costal junction.

The ossification groove (Ranvier's *encoche d'ossification*) of the radius of a 75-day old rabbit contains a bony lamella (fig. 1) which is, of course, the section of a ring presenting two faces and two edges. Its central face is in close contact with the growth cartilage and the most peripheral trabeculae of endochondral bone. Its peripheral face is separated from the periosteum by a thick layer of periosteal cells. Its epiphyseal edge is free and shows signs of intense osteogenesis. Its diaphyseal edge is also free and is being resorbed by osteoclasts. Growth and resorption are going apace for the height of the ring remains approximately the same during the development.

Figure 2 represents the chondro-costal junction of a 42-day-old rabbit. It shows the same bony ring as the radius.

This ring we will call the "perichondrial ring of the ossification groove"; the expression is somewhat lengthy but it has the advantage of being self-explanatory.

Almost any other growing extremity of a long bone might have been chosen to show the ring described and it is surprising indeed that such a structure could thus far have been overlooked.

The formation of the perichondrial ring is easily understood if one follows the growth of the primitive bony tube.

In its earliest stage it is continuous from end to end, but after a while, its extremity is cut off from the middle part by osteoclasts and becomes the perichondrial ring. The faster the growth of the cartilaginous epiphysis, the sooner the extremity of the tube is isolated. From that time on the extremity of the shaft is made of two elements: one is the perichondrial ring of periosteal origin, the other is the endochondral trabeculae which merge into the peripheral parts of the growth cartilage and are continuous with the middle part of the shaft. This is clearly shown by figures 1 and 2; it would appear still more clearly on transverse sections. It goes without saying that, if followed towards the diaphysis, the endochondral bone is seen to be transformed gradually into periosteal bone.

This evolution of the diaphysis has been studied more thoroughly in a recent paper (Lacroix, '44). The point which must be stressed for the present investigation is this. The perichondrial ring of the ossification groove is the extremity of the primitive diaphysis which has been cut off from the other parts of the bone and continues its own evolution.

EXPERIMENTS

The data submitted for discussion are almost entirely supplied by the results of transplantation experiments in which the central part of a growth cartilage has been grafted in different organs and its evolution studied by histological examination.

1. In a first group of experiments, the central part of the distal cartilage of the radius has been grafted into the brain.

In a 9-day-old rabbit this cartilage was cut off with a sharp knife by two transverse and parallel sections; its whole peripheral region was then taken away by four sections at right angles. The disc-shaped growth zone was thus reduced to a small cubic piece consisting exclusively of cartilaginous tissue without any remnant of the ossification groove. A hole was drilled in the parietal bone of a rabbit of the same litter and the graft was pushed into its brain.

One month later the graft was recovered and studied in serial sections.

It had produced a large quantity of endochondral trabeculae but there were being resorbed and replaced by hemopoietic marrow. All around the reserve zone of the cartilage (fig. 3), cells which seem to be ordinary fibroblasts had gathered. They had come from a thin membrane surrounding the graft. Some of the cells had taken part in the formation of a thin bony lamella appearing between the cartilage and the enveloping membrane. The bony lamella was growing by its epiphyseal edge and was being resorbed by its other edge. It was similar in all respects to the normal perichondrial ring of the ossification groove (fig. 1).

2. The same kind of experiment was performed using the medullary cavity of the tibia instead of the brain. Here too (fig. 4), 1 month later, the cartilage was found to have grown quite readily. A perichondrial ring had been formed with the participation of osteoblasts supplied obviously by a surrounding membrane which was but a condensation of the medullary tissue.

3. The same result was obtained when grafting the central part of the distal cartilage under the capsule of the kidney. When the grafted cartilage was in contact by its side with the kidney capsule a typical perichondrial ring differentiated itself between the cartilage and the capsule. The latter assumed in this case the structure and function of a normal periosteum.

In another paper ('43) the author has already made a brief reference to this phenomenon (fig. 6 of said paper) and, incidentally, it is this observation which led him to the present study.

The technique of grafting under the kidney capsule was devised by Buyse ('33). Its use supplied the best collection of successive stages. Their analysis shows that the cartilage grows exactly as if it were in its normal place for some time before the bony ring appears.

4. Growth cartilages of the limbs were not the only ones to be tried; ribs were also very easy to handle. In 15-day-old rabbits, the cartilaginous rib adjoining immediately the bony rib was resected on a length of 5 mm., stripped of all its peripheral region and grafted into the medullary cavity of the tibia in an animal of the same litter.

As early as 11 days later a perichondrial ring had formed around the growth zone of the cartilage (fig. 5). The result, however, was not exactly the same as those so far described: in this experiment the ring had differentiated without any connective tissue membrane which might be likened to a periosteum.

5. Another similar observation is worth recording.

A thin plate of elastic cartilage, 10 mm. long and 3 mm. broad was cut off from the ear of a 15-day-old rabbit. At the same time the proximal part of the tibia of the same animal was incised along its longitudinal axis to a depth of a few millimeters. The auricular cartilage was then slipped between the edges of the incision in such a manner that it pierced the center of the proximal growth cartilage. Now, the latter, in spite of its having been wounded, went on proliferating.

In the experiment under consideration, a small part of the growth cartilage became united to the graft; consequently while the larger part was shifting along the graft, the part which was attached to the graft had to remain at the original level. The growth cartilage became distorted and its adherent part soon lost its connections with the main part which grew away.

In some ways the experiment might be considered as an autotransplantation of a piece of growth cartilage in the bone marrow.

Forty-two days after the operation, a perichondrial ring had developed on the flank of the cartilage (fig. 6). The point is this: just as in the rib grafting experiments, the bony ring had been formed without any connective tissue membrane against it; its osteoblasts had come from the surrounding bone marrow.

6. Since it has been shown that some tissues may, after they are dead, retain their inductive properties, it was necessary to repeat one of our experiments with dead cartilage. The central part of a growth cartilage was left to dry at room temperature for 48 hours and then grafted under the kidney capsule. No suggestion of any bony ring was found in the sections.

DISCUSSION

The six groups of experiments demonstrate that the cartilaginous portion of the growth zone is able when alive to induce the formation of a perichondrial ring of the ossification groove.

It remains to be seen whether this conclusion may be extended to normal circumstances.

It was pointed out in the first paragraph that the perichondrial ring is the extremity of the most primitive form of the diaphysis.

On the other hand it is hardly necessary to recall to mind that the middle part of the cartilaginous rod proliferates in two opposite directions on either side of the hypertrophied cells, even before their invasion.

Therefore it seems to be beyond doubt that the formation of the early diaphysis depends directly or indirectly on the activity of the growing cartilage.

It has nothing to do whatever with any muscular activity as supposed by Carey ('20). Moreover the functioning of the growth cartilage appears to be the primary phenomenon and cannot be related to the presence of the diaphysis (Weidenreich, '30; Policard, '41) or to the periosteal ossification (Studitsky, '34 and '36).

During the whole period of growth the cartilage continues to govern the evolution of its perichondrial ring. Besides casting a new light on the origin of the diaphysis the experiments present thus a wider interest since they show that an induction phenomenon may be active at a later stage than it is generally thought.

There is a difference though between the normal mechanism and some experimental data. Under normal circumstances the osteoblasts of the perichondrial ring are undoubtedly supplied by the periosteum. In the experiments these osteoblasts may appear not only without any periosteum but even without any connective tissue membrane at all. Consequently it must be said that the periosteal origin of the bony ring is merely incidental and cannot be related to any intrinsic property of the periosteum.

The expression "perichondrial ring" is thus the best suited since it does not imply hypothesis. Should the expression "periosteal ring" be preferred, it must be kept in mind that, however right for the normal development, it seems to take for granted the existence of a mechanism which it is by no means necessary to postulate.

These considerations should not be misunderstood; they are not intended to deny the periosteum its well known properties. Contrary to Leriche's ('39) contentions the author has repeatedly observed that, without the tiniest bony spicule, grafted periosteum is able to produce bone. The origin of the osteogenic properties of the periosteum thus still remains obscure. This subject is now being investigated.

SUMMARY

1. The ossification groove of a growing long bone contains a perichondrial bony ring of periosteal origin. This ring represents the extremity of the primitive diaphysis which has been cut off from the middle part of the bone at an early age.

2. In rabbits the central part of the growth cartilage of the distal radius or of the rib has been grafted into the brain, the medullary

cavity of the tibia and under the kidney capsule. Under these circumstances a bony ring identical to that of the ossification groove appears around the cartilage.

3. The formation of the primitive diaphysis and, subsequently, of the perichondrial ring appears as an induction phenomenon directed by the growing cartilage.

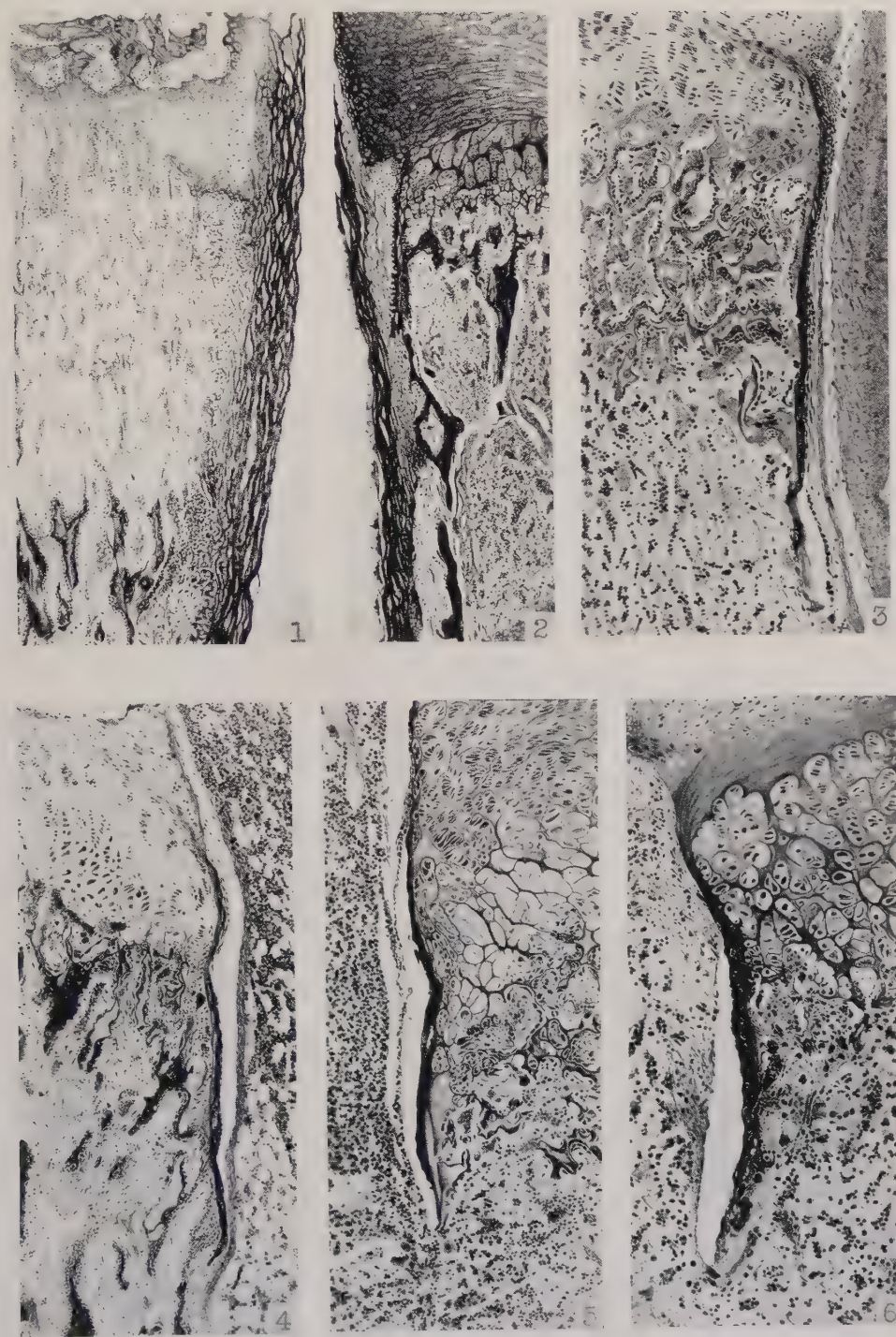
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PLATE 1

EXPLANATION OF FIGURES

- 1 Distal extremity of a radius of a 75-day-old rabbit. Longitudinal section. Perichondrial ring. $\times 49$.
- 2 Chondro-costal junction of a 42-day-old rabbit. Longitudinal section. Perichondrial ring. $\times 34$.
- 3 Homo-transplantation of the central part of the distal growth cartilage of the radius in the brain. Result 1 month later. Perichondrial ring. $\times 70$.
- 4 Homo-transplantation of the central part of the distal growth cartilage of the radius in the medullary cavity of the tibia. Result 1 month later. Perichondrial ring. $\times 62$.
- 5 Homo-transplantation of the central part of the growth cartilage of a rib in the medullary cavity of the tibia. Result 11 days later. Perichondrial ring. $\times 90$.
- 6 Auto-transplantation of the central part of the proximal growth cartilage of the tibia in the medullary cavity of the same bone. Result 42 days later. Perichondrial ring. $\times 120$.



THE ORIGIN OF THE CILIARY GANGLIA IN THE CHICK EMBRYO

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ONE PLATE (FIVE FIGURES)

INTRODUCTION

The literature on the development of the cranial autonomic ganglia is continually increasing. Investigators study a series of embryos using a newer stain or different techniques and on the basis of their observations describe the origin and development of these ganglia. The results are quite uniform in their variability. The cells which develop into the cranial autonomic ganglia come either from the brain stem, migrating along the motor nerves; or they come from the sensory ganglia, migrating along the sensory nerves, or they come from both. The variability and the abundance of the reports on this subject make it quite obvious that the facts cannot be determined by studying normal embryos. The methods of experimental embryology should provide evidence which is reasonably convincing.

For example, if the ciliary ganglion is derived from the cephalic neural crest which gives rise to the trigeminal ganglion, then the removal of the anlagen of the fifth ganglion should prevent the development of both the ciliary and trigeminal ganglia.

If the ciliary ganglion arises from neuroblasts which migrate from the midbrain along the oculomotor nerves, then removal of the midbrain before the migration occurs should prevent the formation of the ganglia. Such experiments are herein reported.

EXPERIMENTS AND RESULTS

In three embryos in a series from which the hindbrain had been removed it was found that the part of the trigeminal ganglion which arises from the neural crest was absent on one or both sides (fig. 1). This was due to the fact that in removing all the ectoderm overlying the hindbrain, together with the underlying neural tube, the neural crest had been removed also. (These operations were designed to eliminate the vagus nerves, Jones, '42). In these embryos the ciliary ganglia were

normal in appearance (fig. 4) even though the major part of the trigeminal ganglia was lacking. In the embryo from which figure 1 is taken that part of the trigeminal ganglion which is derived from the neural crest is not present. The placodal part of the ganglion is represented by a small group of cells located posterior to the developing eye and a few scattered cells slightly medial and posterior to this ganglion. So, it is evident that the ciliary ganglia do not develop from the cephalic neural crest which gives rise to the major part of the trigeminal ganglion. Later it will be shown that the placode which contributes to the formation of the trigeminal ganglion is not the anlagen of the ciliary ganglion.

In a series of embryos I attempted to remove the midbrain to see if the cells of origin of the ciliary ganglion would be eliminated. This procedure seemed fairly easy to accomplish as far as could be judged while performing the operation, but study of the embryos from 2 to 5 days postoperatively demonstrated that it was a difficult thing to do. Nervous tissue nearly always developed in the ventral part of the field. It was not difficult to eliminate the oculomotor nerves but their elimination did not prevent the development of the ciliary ganglia (fig. 3), which had no connection with the brain. Although the midbrain was removed in several dozen embryos, and although the oculomotor nerves were eliminated by this procedure, the ciliary ganglia always developed after such an operation.

Since the ciliary ganglion is closely related physiologically and anatomically to the eye the possibility was considered that the ganglion might develop from the optic stalk. Three embryos from which the optic stalk was removed unilaterally at 42 hours incubation subsequently showed the absence of one eye but normal ciliary ganglia on both sides. Thus the ganglion does not develop from the optic stalk.

Accordingly in another series of embryos that part of the forebrain between the optic stalks was removed together with the midbrain. Most of these embryos were subsequently too deformed to be valuable for study, but three of them had fairly well developed eyeballs so that serial sections could be studied intelligently. These three had no ciliary ganglia. One cannot say from this evidence what is the exact location of the cells which form the ciliary ganglia, but it is logical to speculate that they are thalamic in origin. In these embryos the trigeminal ganglia were present since neither the neural crest or the placodes were removed. Thus in these embryos the ciliary ganglia failed to develop despite the presence of the cephalic neural crests and the placodes.

DISCUSSION

From the above data it is evident that the ciliary ganglion does not develop from the neural crest, or from ectodermal placodes; that although the ganglion is intimately associated with the oculomotor nerve in its normal development yet the ganglion can develop independently of the oculomotor nerve; and that the cells of origin may arise from the forebrain. The knowledge of the exact origin of these cells must depend upon further experiments.

That the ciliary ganglion arises from the neural tube rather than from the neural crest is consistent with the development of other parts of the autonomic system. For it has been demonstrated that the sympathetic ganglia of the sympathetic trunk arise from the neural tube, and that the visceral ganglia of the thorax and upper digestive tube arise from the hindbrain (Jones, '37, '39, '41, '42).

Numerous authors have described cells in the oculomotor nerves which were assumed to be destined for the ciliary ganglion. A very extensive and detailed study of the development of the oculomotor nerve and ciliary ganglion in the chick was reported by Carpenter in 1906. He described neuroblasts which migrated from the midbrain to the ciliary ganglion along the third nerves and also neuroblasts which migrated from the trigeminal ganglion along the ophthalmic nerve. At that time it was generally accepted that the autonomic system was derived from the neural crest, so Carpenter postulated that these cells of mid-brain origin were neither autonomic nor cerebrospinal but a special motor element associated with the great specialization of the intrinsic muscles of the avian eye. He did state, however, that some authors, notably Harrison, had suggested that there was possibly a contribution to the autonomic system from the neural tube and that later studies might prove this to be true.

Carpenter's description of the contribution from the trigeminal ganglion is very important because it helps to reconcile conflicting data, pertaining to the origin of the ciliary ganglion. He reports that a large number of cells migrate from the trigeminal ganglion along the ophthalmic nerve, but that only a few reach the ciliary ganglion. Many migrate past the ramus to the ciliary ganglion and form a fusiform ganglion between the forebrain and the eyeball just caudal to the laterally projecting hemisphere. This ganglion develops to a size of 165 by 75 micra in the 88- to 97-hour chick. By 100 hours this ganglion has begun to disappear and is completely gone by 120 hours. Ganglion cells are present here and there along the nerve but most all of the cells which

migrated from the trigeminal ganglion have disappeared by 120 hours. Carpenter suggests that these cells may have a phylogenetic significance. Probably these migratory and transitory neuroblasts are the cells that have been so often assigned a role in the formation of the ciliary ganglion.

Cowgill and Windle ('42) have described neuroblasts along the ophthalmic nerve between the trigeminal and the ciliary ganglia in the cat embryo. Similar cells were not discovered in the oculomotor nerve. Therefore, they conclude that the ciliary ganglion is derived entirely from the trigeminal ganglion. They think that the cells in the oculomotor nerve which have been considered neuroblasts by Carpenter ('06), Kuntz ('20), Mihalik ('40) and Jones ('42) are not nervous elements because they cannot be stained with the same technique that successfully delineates neuroblasts in the ophthalmic nerve.

That there are cells of neural crest origin along the ophthalmic nerve is unquestioned. That these cells are the progenitors of the ciliary ganglion is disproved by the fact that the ciliary ganglia develop normally following removal of the trigeminal neural crest. This fact has been reported recently by Yntema ('44). Cowgill and Windle report that attempts to remove the neural crests have not been successful in their laboratory and state that it is difficult to be certain that crest remnants do not remain. Similar difficulties have not been encountered in this laboratory and the presence or absence of the semilunar ganglion can always be ascertained. At any rate the difficulty of the procedure should not preclude a logical conclusion from whatever data are obtained.

There are several possible explanations as to why Cowgill and Windle could not find neuroblasts in the oculomotor nerve. First, they assumed that cells in the motor nerves would have the same staining reactions as neural crest cells. In using the Cajal technique extensively in studying chick embryos, I have observed that it delineates dorsal root ganglion cells clearly but is entirely inadequate in staining neuroblasts in the ventral roots of spinal nerves or in the myenteric plexus of the digestive tube. Very likely the pyridine silver technique is similar to the Cajal technique in this respect. Also, Cowgill and Windle state that a neuroblast must have neurofibrillae before it can be considered as such. Perhaps it may be questioned whether this criterion is warranted. For example, the cells in the oculomotor nerve roots of normal chick embryos which appear similar to the cells in the midbrain (figs. 4 and 5) are very immature and undifferentiated and even contain

a mitotic figure. Would one expect neurofibrillae to be present in a neuroblast still capable of mitotic division? Also, there are no similar cells in the connective tissue surrounding the nerve. Therefore it would appear reasonable to consider that the cells in these oculomotor nerves (figs. 2 and 5) are neuroblasts derived from the brain.

Another possible explanation of why Cowgill and Windle were unable to identify neuroblasts in the oculomotor nerve of their embryos is that in mammals the gestation period is longer and the migratory cells do not move in such large numbers as in the chick. In the human embryo very small numbers of cells are seen at any one time in the ventral roots of the spinal nerves.

But, as was pointed out in the beginning, examination of normal embryos is not likely to yield convincing evidence of the origin of the ciliary ganglion. The experimental evidence herein presented permits no other inference than that the ciliary ganglion is formed from cells originating in the brain and not from the neural crest, or specifically from the trigeminal ganglion. These cells may or may not migrate along the fibers of the oculomotor nerve, but it appears that they do in normal embryos, and that they are derived from some part of the forebrain rather than from the midbrain. This evidence adds support to the general concept that the ganglia of the autonomic nervous system arise from cells which migrate from that area of the central nervous system that furnishes preganglionic fibers to these ganglia.

SUMMARY

Removing the cephalic neural crest which gives rise to the major part of the trigeminal ganglion does not interfere with the development of the ciliary ganglion in the chick embryo. Removing the midbrain prevents the formation of the oculomotor nerve but does not prevent the development of the ciliary ganglion. Removal of the midbrain and the forebrain between the optic stalks prevents the formation of the ciliary ganglia. Therefore, it is concluded that the ciliary ganglia develop from cells which migrate from the forebrain.

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PLATE 1

EXPLANATION OF FIGURES

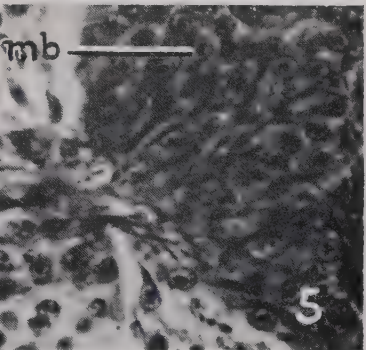
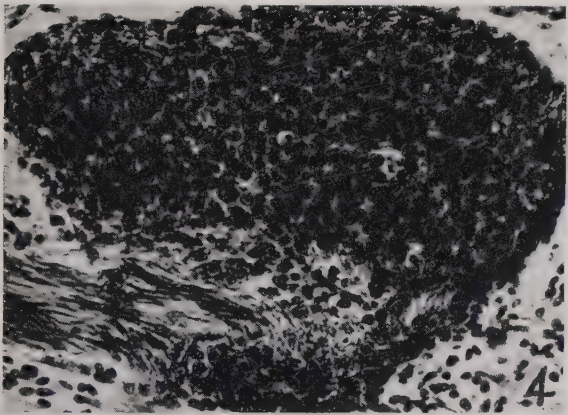
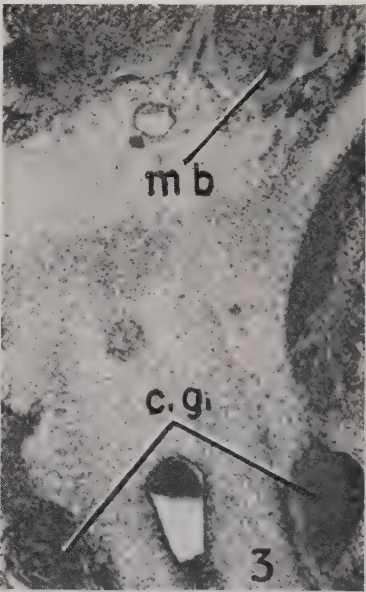
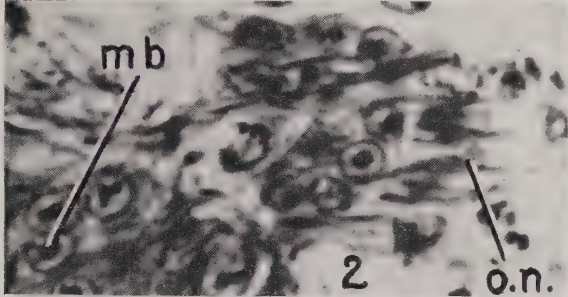
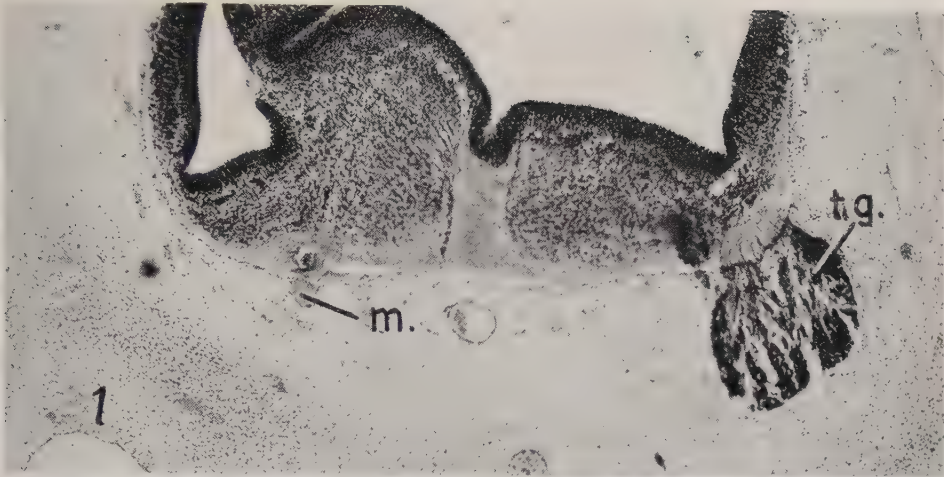
1 Section through pons of 6-day chick embryo from which cephalic neural crest was removed on one side at 40 hours. t. g., trigeminal ganglion; m., motor division of trigeminal nerve.

2 The beginning of an oculomotor nerve in a 75-hour chick embryo; mb., midbrain; o.n., oculomotor nerve.

3 Section of a 7-day chick embryo from which the midbrain was removed at 40 hours of incubation. No oculomotor nerves are present. mb, midbrain remnant; e.g., ciliary ganglia.

4 Ciliary ganglion in a 6-day chick embryo from which the cephalic neural crest was removed at 38 hours of incubation.

5 The oculomotor nerve leaving the midbrain in a 75-hour chick embryo. mb, midbrain; o.n., oculomotor nerve.



THE FIRST MATURATION DIVISION OF THE RAT OVUM¹

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TWO PLATES (TWELVE FIGURES)

The studies of Van Beneden (1875), on the maturation division of the rabbit ovum, laid the foundations for similar investigations in other mammals. Since this early investigation major contributions to the problems of polar body formation were made by Sobotta (1895) and Kirkham ('06), in the mouse; Moore ('08), in the guinea pig; Van der Stricht ('23), in the bat; Sobotta and Burckhard ('11), and Kirkham and Burr ('13), in the rat; Longley ('11), in the cat; Hartman and Corner ('41) in the monkey; Thomson ('19), Hoadley and Simons ('28), and Rock and Hertig ('44) in the human.

Many of the earlier investigators were handicapped in obtaining material during the interval of first polar body formation because the temporal relationships of the various phenomena of the mammalian reproductive cycle were not clearly understood.

In Sobotta and Burckhard's extensive study on the formation of the polar body in rat ova, a description is given of only one ovarian egg in the process of first maturation division. The infrequent observations of first polar body formation led these authors to conclude that the whole process must be consummated in a very short period of time.

Maturation figures have been described in atretic follicles of various sizes and in ovaries excessively stimulated by hypophyseal hormones (Dempsey, '39; Guthrie and Jeffers, '38). Illustrations of the process of first polar body formation in normal follicles are only sparsely distributed in the literature and these in the form of drawings or sketches.

In previous studies on the changes in the ovaries of rats during heat (Blandau and Soderwall, '41; Blandau and Money, '43), ovaries were obtained which contained ova in various stages of first polar body formation. From these, those polar bodies that were completely contained within a single 10 μ section were selected for the present study.

¹ This investigation was supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council.

Sufficient stages were available to permit the establishment of the approximate sequence of events of the first polar body formation.

MATERIALS AND METHODS

The ovaries which constitute the material for these observations were accurately timed with respect to the estrous cycle by the method of manual manipulation described by Blandau, Boling and Young ('41). It has been shown, in the rat, that at the beginning of heat an eightfold acceleration of follicular growth begins. This rate of growth is maintained throughout the heat period and terminates in ovulation (Boling, Blandau, Soderwall and Young, '41). It is during this accelerated period of follicular growth that the first polar body is formed.

All of the ova to be described were found in follicles the volumes of which ranged from 210 to $360 \times 10^6 \mu^3$, a size determined by Boling, Blandau, Soderwall and Young ('41) to be within the range of size of follicles within the period of preovulatory swelling. Definite cytological changes normally associated with the preovulatory growth period further attested to the maturity of the follicles.

The ovaries were fixed in Bouin's fluid, dehydrated in dioxan, embedded in paraffin, sectioned serially at 10μ and stained in Delafield's hematoxylin and eosin. Photomicrographs of the polar bodies were made at a magnification of $480 \times$.

OBSERVATIONS

The first maturation spindle to be described (fig. 1) was found in an ovary removed 4 hours after the onset of sexual receptivity. The spindle is cut approximately along its longitudinal axis and the greater portion of the spindle is confined to the one section. The spindle occupies a paratangential position in relation to the surface of the ovum. It is quite short and broad, and there are well defined spindle fibers. As was earlier shown by Kirkham ('06) there are no centrioles in the meiotic spindle of the rat and, as a result, the spindle fibers do not converge to a sharp focus. Of interest is the fact that at this stage the elongated chromosomes may be seen migrating to the poles. This is the only specimen in which chromosome migration was in progress at the time of fixation. A somewhat similar stage is sketched by Moore ('08) in his study on the maturation of the guinea pig ovum.

It is generally agreed that the first maturation spindle discharges its function speedily and is only rarely observed in normal follicles. The rotation of the spindle on its long axis must take place very rapidly

since all of the polar bodies observed in the stage of abstriction lie in a more or less radial position.

The ova shown in figures 2 through 8 were observed in ovaries removed approximately 4 hours after the onset of heat. In figures 2, 3 and 5 the polar spindles have assumed radial positions and the chromosomes are clumped in solid masses at the poles. The spindle fibers and mid-bodies are clearly discernible. The polar bodies are approximately spherical in shape and their peripheral zones are composed of a clear, homogeneous protoplasm. Very little if any of the granular material of the egg protoplasm passes into the polar body. The darker central zone appears to be composed chiefly of spindle fibers. These eventually lose their identity as fibers and the central zone then presents a very finely granular protoplasm which stains slightly basophilically.

Just beneath the site of the mid-bodies is the point at which abstriction occurs (figs. 3, 4 and 5). The mid-bodies lose their identity very soon after the polar bodies are formed. They are rarely observed in polar bodies in which abstriction has been completed.

Polar bodies which have undergone complete abstriction are shown in figures 4 through 8. The peripheral zone of clear cytoplasm is distinctly visible. The aggregated chromosomes within the polar bodies may either form a single compact bar, or be broken into a number of smaller aggregations. The chromosomal mass left behind in the ovum at first forms a tightly packed, spherical mass in which separate chromosomes are not distinguishable. This condition, however, is only transient and the chromatin material soon begins to separate into distinct globular units (fig. 11). After the polar body has separated from the ovum a few spindle fibers may still be seen radiating toward the surface of the ovum but these soon disappear (figs. 5, 7 and 8).

Ova in which the first polar bodies have also undergone complete abstriction are shown in figures 9 through 11. These ova were observed in ovaries removed approximately 6 hours after the onset of heat. Special attention is called to the position of the chromatin masses remaining within the ova. In all three cases the chromatin masses are confined to definite hillocks which extend for some distance above the egg's surface. This arrangement is similar to that figured by Van der Stricht ('23) for the first maturation divisions in several guinea pig and bat ova and probably represents variations in the normal maturation process.

The ovum shown in figure 12 was removed from an animal approximately 6 hours after the onset of heat. Microscopic examination reveals a barrel shaped spindle with definite spindle fibers running tangentially to the surface of the egg. The majority of the chromosomes are confined to the two poles but there are several chromosomes which as yet have not completed this migration. An almost identical figure is described by Moore ('08) in a first polar body of the guinea pig.

SUMMARY AND CONCLUSIONS

Photomicrographs and descriptions of a series of selected sections fortunately including most or all of the first polar bodies of rat ova are presented.

1. The first maturation spindle is short and broad with no observable centrioles.

2. The maturation spindle at first occupies a paratangential position in relation to the surface of the egg. Chromosome migration from the metaphase plate to the poles is in progress before the spindle rotates on its long axis and assumes a more or less radial position.

3. Abstriction of the polar body occurs just below the region of the mid-bodies and as a result they are included in the polar body protoplasm.

4. The protoplasm of the first polar body is divided into two zones: a peripheral zone which is clear and homogeneous and a central zone which is composed of a finely granular protoplasm.

5. Variations in the surface of the ovum at the site of polar body formation are described.

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PLATE 1

EXPLANATION OF FIGURES

1 through 6 First maturation spindles and polar bodies observed in ovaries removed approximately 4 hours after the onset of sexual receptivity. $\times 480$.

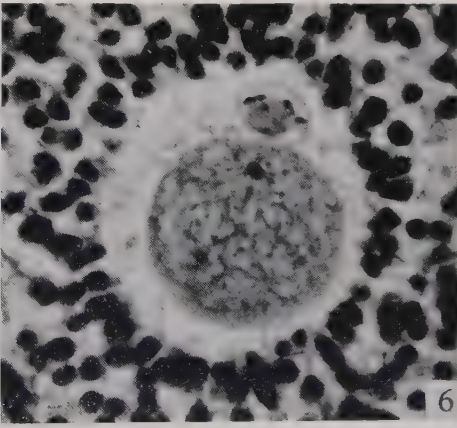
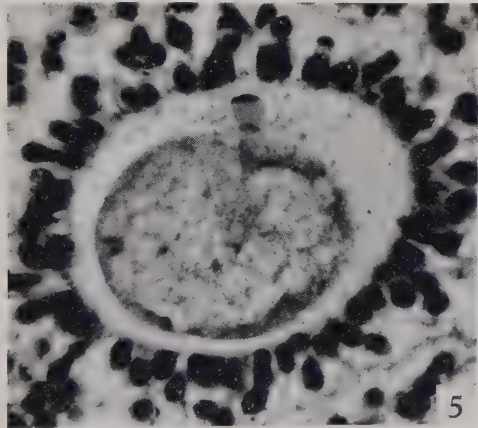
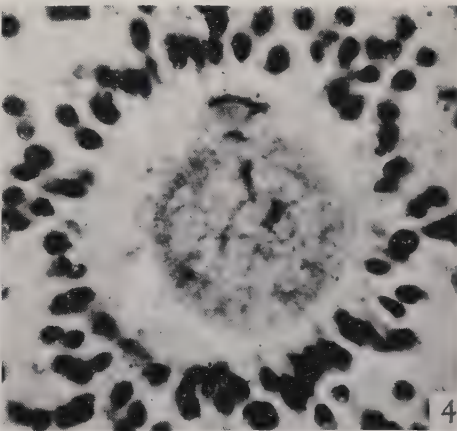
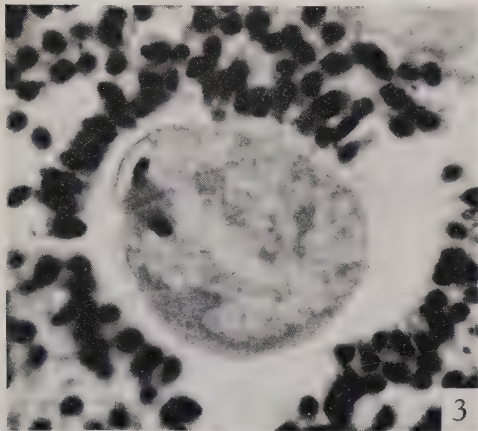
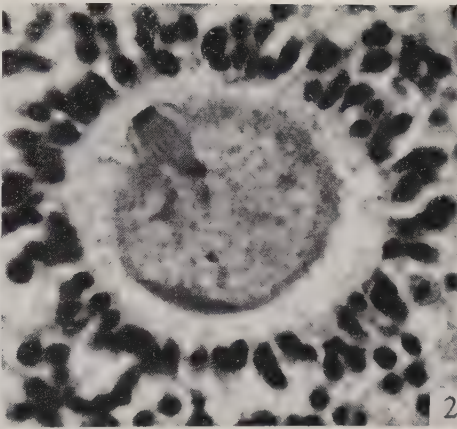
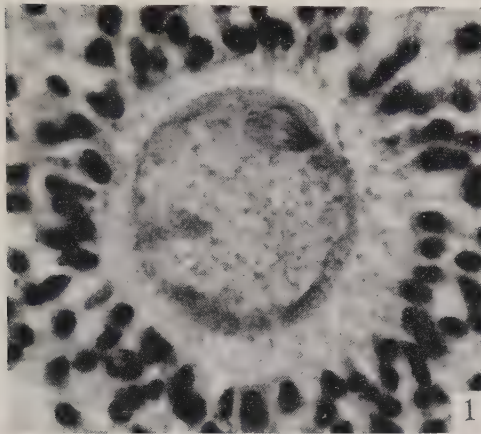
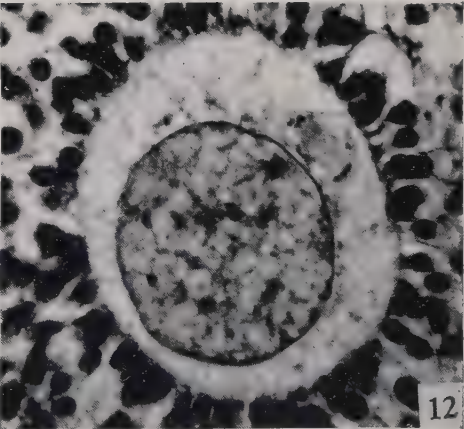
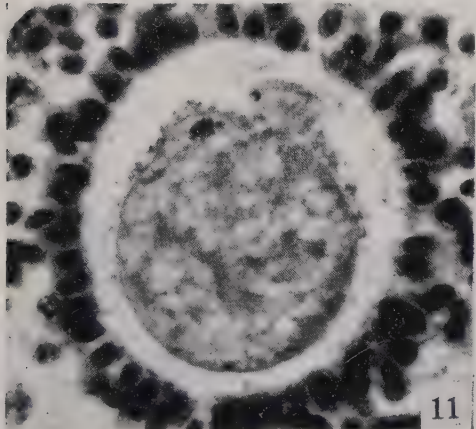
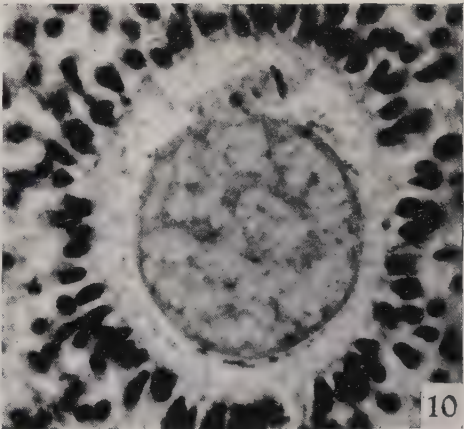
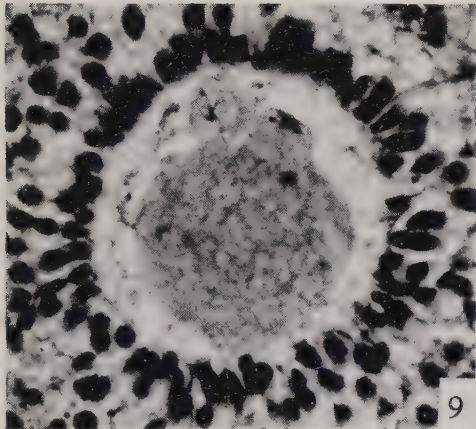
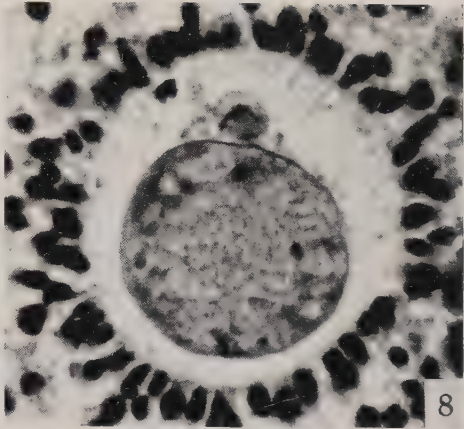
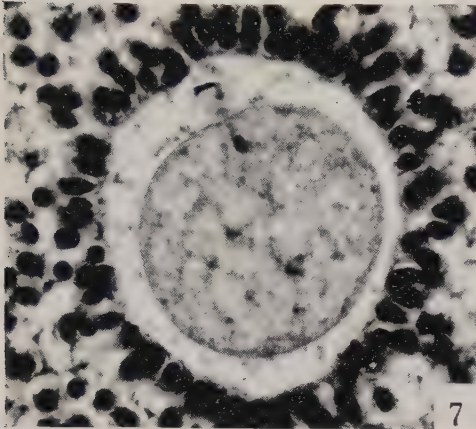


PLATE 2

EXPLANATION OF FIGURES

7 through 12 First polar bodies observed in ovaries removed approximately 6 hours after the onset of sexual receptivity. $\times 480$.



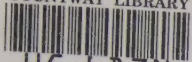
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